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P. J. du Toit,
Director of Veterinary Services.

IMPORTANT NOTICE TO SUBSCRIBERS.

War-time conditions have inevitably caused irregularity in the times of appearance of the "Onderstepoort Journal", and the original plan of publishing annually four separate numbers (in two volumes) has had to be abandoned temporarily.

Delays in printing have resulted in discrepancies between the dates appearing on the volumes and the actual dates of publication. Thus Volume 17 bore the date "July and October, 1941", although it did not appear until 1943. In order to bridge the gap, the present volume (18) is dated (correctly) "July and October, 1943". Efforts will be made to keep up to date in future, even though this may mean issuing smaller volumes.

Subscriptions entered for 1942 will be credited for 1943.

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A Simple Insect Cage-Olfactometer.

By G. A. HEPBURN, Division of Entomology, Pretoria.

THE difficulties surrounding studies on the chemotropic behaviour of insects conducted under field conditions have been appreciated by many entomologists. The conflicting factors ever present in such work often prevent a clear picture of the insects' reactions being obtained. The desirability therefore, of studying the behaviour of insects under laboratory controlled conditions has been stressed by many investigators. This applies very notably to the sheep blowfly problem involving as it does an understanding of the olfactory responses of the several species of flies.

The object of this paper is to describe for the benefit of other workers a simple type of cage-olfactometer which, with modifications may be used for studying the olfactory responses of various species of insects.

While many olfactometers have been devised their manipulation often demand so much preliminary preparation that, for testing large numbers of chemicals too much time is required. The writer f els therefore that, for the examination of a large number of chemicals for attractiveness or otherwise as simple a device as possible should be used.

The apparatus now being used for olfactory studies of sheep blowflies, particularly *Lucilia cuprina* Wied., has been evolved from one originally described by Ripley and Hepburn (1929) in their work on Natal fruit fly (*Pterandrus rosa* Ksh.). This was subsequently improved by them for the same investigation but it was never described. The apparatus in its present form differs in some mechanical features from its undescribed prototype.

The original apparatus was designed primarily to obtain rapid results on the olfactory quality of a large number of substances to fruit flies (*P. rosa* Ksh.) and, in the present investigation, the writer is using it in a similar connection with sheep blowflies particularly *L. cuprima* Wied.

Description of the Apparatus.

The apparatus consists essentially of a fly container or cage and two lateral trapping units carrying the odoriferous substances. The cage (Fig. 1) is made of a wooden framework 35 cm. high, 30·5 cm. deep, 30·5 cm. wide, which is covered with cloth gauze. Cloth gauze is used in preference to wire mesh because flies damage themselves by flying against wire mesh and there is a tendency for toxic substances to be produced by flies salivating or defecating on the metal. A cloth gauze sleeve (*a*) (Fig. 1) is sewn onto the covering of the trap in the mid front of the cages. Through this the flies are introduced to the cage and also a Petri-dish (*b*) containing a cotton pad soaked in water. 13 cm. from the top of the cage on either side is a wooden bar (*c*) in the centre of which is a hole (*d*) 2·2 cm. in diameter through

which the flies can pass into the trap or catching unit (*e*) (Fig. 2). The size of this aperture is determined very greatly by the type of insect being studied. It has been found that, with a vigorous and active insect like *L. cuprina*, the aperture must be small in order to reduce random catching as much as possible. The middle portion of each of these cross bars has been shaped so that it shields the mouth of the trap thus allowing the greater portion of the odour within the unit to escape through the entrance hole into the cage, while a relatively small amount escapes around the edges. In addition, any reflection from the glass traps is greatly minimised from shining into the cage, thereby reducing the possibility of creating a visual attraction to the flies. In early experiments with this apparatus the traps were covered on the tops and bottoms with brown paper, but later this was discontinued as it apparently made no difference to the results.

Fig. 1

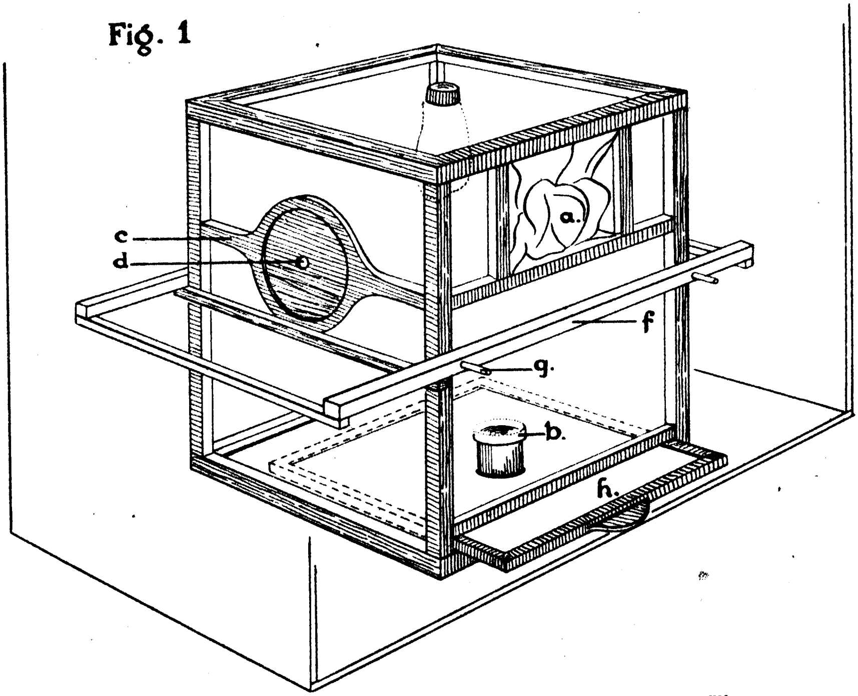


FIG. 1.—Diagram of cage-olfactometer set up in a compartment. The gauze covering is not shown. The trough is shown projecting; in the course of an experiment this is closed. The traps (Fig. 2), when in use, are placed on the supporting frameworrk f on etiher side to cover d. (Drawn by Miss G. E. Laurence.)

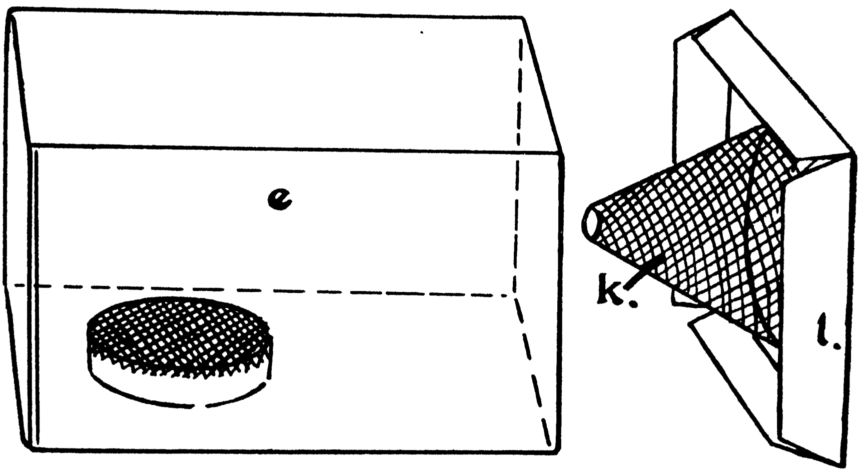
There are two traps which are placed one on each side and supported by means of an outer wooden framework (*f*. Fig. 1), 56 cm. long and 34·5 cm. across, fastened to the cage by four removable pegs (*g*). The traps rest horizontally on this framework with the open ends in contact with the cross bar (*c*).

On the base of the cage is a removable tray (*h*) made of plywood. This enables one to remove the dead flies. The bottom of the tray is covered with a loose sheet of white blotting paper. In the centre of the tray is a Petri-dish containing a pad of cotton wool soaked in water and from the centre of the top of the cage a strip of cotton wool soaked in water, is suspended.

Glass specimen jars (e. Fig. 2.) 14·2 cm. long, 10·5 cm. wide, and 8·4 cm. deep, are used as traps or fly catching units. The mouths of the jars are fitted with removable metal lids (*l*) into which have been fastened wire mesh cones (*k*) the apices opening within the traps. The basal diameter of each cone is 6·5 cm., the height 6·7 cm. and the diameter of the hole in the apex 0·8 cm. The cone must be fitted so that the apex is opposite the centre of the entrance hole (*d*. Fig. 1) to the trap. The corners of the metal lids are cut away so that the edges may be bent inwards when necessary to increase the tension thus permitting them to fit closely to the glass traps.

The odoriferous substances to be tested may be put into small Petri-dishes or glass weighing bottles or any suitable container. The containers must be screened with a mesh cover to prevent the trapped flies having access to the chemicals. Especially in the testing of liquids it is undesirable for the flies to get into them or to feed on them. The presence of flies in a bait may conceivably introduce upsetting olfactory factors. Furthermore, if the flies can feed readily on the chemical they may thus set up certain stimuli which might give a result not necessarily an olfactory one.

Fig. 2.



The apparatus is set up in a compartment in a dark room the temperature and relative humidity of which can be regulated. It would be an advantage if the dark room could be air-conditioned as this would prevent air pollution by gases given off from the chemicals in the traps. If several cages are to be used simultaneously they should be put in separate compartments in the dark room. The compartments can be made by dividing up a wall bench with asbestos boards or other suitable material. The walls and roof of each compartment should be uniform in colour preferably with a non-reflecting surface. The open fronts of the compartments are fitted with roller blinds which are drawn during the experiment. If possible, the blinds should be opaque having the inner surface the same colour as the inside of the compartment. Each compartment is illuminated by means of a frosted vacuum electric light (25 Watts) suspended 35 cm. above the top of the cage. A vacuum bulb is used in preference to a gas filled one for it produces relatively little heat.

Care must be taken to centre the cage in the compartment so that the intensity of the light is equal on either side. A convenient size for the compartment is 70 cm. wide, 70 cm. deep and 90 cm. high. In preliminary experiments with this apparatus two electric lamps, one on each side, were used but there seemed no advantage in this as one central lamp sufficed.

THE MANIPULATION OF THE APPARATUS.

The condition of the material.

It has been found that *L. cuprina* flies react more readily in this cage olfactometer if they have been kept previously for two days at 25° C. and at a minimum relative humidity of 33 per cent. Twenty-four hours before the flies are to be used in the cage olfactometer their food is removed but they are supplied with a liberal quantity of drinking water all the time.

It has been noted that flies recently fed on meat juices do not react readily when exposed in the cage olfactometer. On the other hand if the flies have been starved excessively their random movements appear to be accelerated.

It is important to adopt a standard procedure in the handling of the flies before they are used in olfactory tests for the behaviour of blowflies is subject to much variation according to their prior treatment.

Numbers of Flies used in each Cage.

A minimum of one hundred flies *L. cuprina* are used per cage for each experiment but two hundred could be used without overcrowding.

Temperature and Relative Humidity of the Dark Room.

The dark room is kept at a temperature of 26-29° C. and a relative humidity of 40 to 50 per cent. The relative humidity within the cage olfactometers is probably somewhat higher owing to the presence of wet cotton pads.

At temperatures of 21° C. and below *L. cuprina* does not react satisfactorily in this apparatus.

Time Required for Experiments.

The duration of any experiment cannot be predetermined for much depends on the attractiveness of the chemicals to be tested. Very definite results have been obtained within fifteen minutes when a highly attractive substance has been used, and at other times an exposure of four hours has been necessary to obtain a result. In general, satisfactory results have been obtained during exposures of ninety minutes for each test.

In each experiment two substances are tested one in the left side trap and the other in the right. At the end of the test the entrance holes in the cage are plugged and the traps removed for the counting and sexing of the flies. The flies are killed in the trap by subjecting them to ether or chloroform vapour; afterwards they are removed for counting. The glass traps, metal covers and cones are ventilated in sunshine for about an hour and the bait containers are cleaned. Fresh bait is put into the containers and the apparatus re-assembled and the test is repeated, but the traps are interchanged, the former left side one being placed on the right and *vice versa*. Each complete unit i.e., glass jar, chemical and lid with wire mesh

cone is reversed in position. In each experiment therefore, four separate catches of flies are obtained and from these figures the relative attractiveness of the two test substances can be calculated. After the conclusion of an experiment the untrapped flies are removed, counted and sexed. The apparatus is cleaned, put out in a sunny place for about an hour and then replaced in its compartment in the dark room.

Particular care must be observed in the cleaning of the glass traps, dishes and cones. The cones are washed in hot water and then placed in a jet of steam for ten minutes. Ordinary washing in cold water fails to remove the odour of beef bait from the wire mesh.

Concentration of Chemicals.

L. cuprina is extremely sensitive to the odours of decomposing meat or carrion. Five drops of beef soup* (0·15 c.c.) diluted in 2 c.c. of water have been found to attract the flies in this apparatus. For most tests however, it has been found that 2·5 c.c. of undiluted soup are required as a standard bait. Chemicals of unknown attractiveness are tested at various concentrations some of them being used undiluted and others in very dilute solutions e.g., 0·0005 per cent. Other factors determining concentrations of the substances depend on the nature of the solvents used.

Testing for Attractant, Repellent and Obscurant Odours.

The apparatus may be utilised for determining whether a substance is an attractant by testing it against a known attractant, or water, or a blank according to circumstances. The obscurant value of any substance can be determined by putting the same attractant in both traps with the chemical added in a separate container to the one side. The practice of pouring a chemical over an attractive bait in an endeavour to measure its obscurant value is to be deprecated. Mechanical and chemical factors are thus introduced which might give rise to misleading results.

If a chemical, when tested against a blank, catches no flies while the latter does, it may be regarded as a repellent.

Results.

The discussion of data obtained with this apparatus does not fall within the scope of this paper but a few results may be mentioned to show the capabilities of this cage-olfactometer.

In the paragraph dealing with the cleaning of the apparatus reference was made to the importance of removing all traces of odours from the traps before commencing new experiments. This was forcibly demonstrated in a test in which a beef bait was run versus an aqueous solution of ethyl sulphide 0·05 per cent., the former caught 24 flies and the latter four in forty-five minutes. The beef bait was removed and replaced by a 0·125 per cent. solution of ethyl sulphide tested against the original 0·05 per cent. solution, but the cone of the trap formerly containing beef was not washed but only aired in the sun for a few minutes. Within ten minutes of the beginning of the test twenty flies were caught in this trap. The experiment

* An equal weight of minced beef and water inoculated with a culture of bacteria from sheep intestines is incubated for 40 hours at 37°C. This is centrifuged for twenty minutes and the clear liquid or beef soup thereby obtained is used as a control bait.

was stopped and the trapped flies returned to the cage. An odour of beef bait clinging to the mesh cone was noticeable. After the cones were thoroughly cleaned the experiment was restarted and the subsequent reactions of the flies were markedly different, only sixteen being attracted to this trap in ninety minutes.

On another occasion duplicate experiments in which a very weak beef bait (0·15 c.c. beef bait soup in 2·35 c.c. of water) was tested against water, were run simultaneously. The results were almost identical the ratio of relative attractiveness between the beef and water being 2·3 and 2·7 and their correction factors for position errors were 1·02 and 1·3 respectively.* A correction factor of 1 indicates equality in attactiveness of the positions of the traps.

A beef bait soup of the concentration indicated is an extremely weak one and the consistent results in the duplicated test show both the precision of the apparatus and the great sensitivity of the flies.

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---

* The above method of comparing two substances was described by Ripley & Hepburn (1929.*b*). The formula used for calculating the relative attractiveness of the two substances and the relative attractiveness of the positions occupied by the traps is derived thus:—

1. Using bait a in positions 1 and 2 (P_1 and P_2)

$$\frac{P_1}{P_2} = \frac{a_1}{a_2} = K.$$

2. Using bait b in positions 1 and 2 (P_1 and P_2)

$$\frac{P_1}{P_2} = \frac{b_1}{b_2} = K \text{ if the situation remains more or less the same.}$$

3. Using bait a in position 1 and bait b in position 2

$$\frac{P_1}{P_2} = \frac{a_1}{b_2} = l.$$

4. Using bait b in position 1 and bait a in position 2

$$\frac{P_1}{P_2} = \frac{b_1}{a_2} = m.$$

$$\text{Now } lm = \frac{a_1}{b_2}\frac{b_1}{a_2} = \frac{a_1}{a_2}\frac{b_1}{b_2} = K^2$$

$$\therefore \sqrt{lm} = K.$$

$$\therefore P_1 = \sqrt{lm}\, P_2.$$

The $\sqrt{lm}$ is thus the correction factor.

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Sheep Blowfly Research I.—A Survey of Maggot Collections from Live Sheep and a Note on the Trapping of Blowflies.*

By G. A. HEPBURN, Division of Entomology, Pretoria.

SMIT and du Plessis (1927) and Smit (1931) have discussed the subject of the distribution of blowflies in the Union of South Africa with special reference to those causing myiasis in sheep. Their results indicated that maggots of *Chrysomyia chloropyga* Wied., and *Lucilia sericata* Meig., were the ones most commonly found and that the two species were about equally abundant. The hairy maggots of *Chrysomyia albiceps* Wied., were much less common on the sheep.

In recent years similar surveys carried out in Australia have proved that *Lucilia cuprina* Wied., a primary blowfly, was responsible for the majority of maggot infestations on sheep. The two species of blowflies *L. sericata* and *L. cuprina* have been thus separated only during the last few years. From the evidence available it is safe to assume that in the literature dealing with the blowflies in South Africa statements on *Lucilia sericata* can be read to refer to the other species *L. cuprina*.

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* Blowflies have been present in South Africa for many years and excellent pioneering work on this group of insects was carried out by Smit and others; but it is only in recent years that these destructive parasites increased to such an extent that they became a serious menace to the sheep and wool industry.

The urgency and seriousness of the problem demanded special measures, and a team of workers was brought together to study the various aspects of the subject. Mr. G. A. Hepburn, B.Sc., was seconded from the Division of Entomology and Mr. M. C. A. Nolte, M.Sc., from the Division of Chemical Services. Other members of the team were Mr. A. H. de Vries, B.Sc., of the Division of Animal and Crop Production and Mr. P. A. Cilliers, B.Sc., whose appointment was made possible by a grant from the Wool Council. The work was carried out under the immediate direction of Dr. H. O. Mönnig, Head of the Section of Parasitology at Onderstepoort. The series of articles here presented contain the first results of this joint undertaking.

Unfortunately the necessity of tackling other urgent problems rendered the continuation of this valuable collaboration impossible. Mr. Hepburn and Mr. Nolte have returned to their former spheres of work, but they have the satisfaction of knowing that they have rendered a lasting service to the sheep and wool industry.

The following articles will indicate the progress that has been made. The complicated interrelation between the different species of blowflies has been studied and deductions of great practical importance have been made. Valuable indications have been obtained regarding attractants and repellents for blowflies. And the success obtained with the remedies recommended for the treatment of myiasis has been most encouraging.

The work is being continued on a reduced scale and further articles in the series will be published.

P. J. DU TOIT, *Editor.*

In the light of Australian experience it was felt advisable to re-survey the position in order to obtain a clear idea of the relative importance and abundance of the species of maggots found attacking sheep. A survey of the main sheep farming areas of each province of the Union was carried out in the manner described by Smit and du Plessis (1927). In addition, two detailed surveys were made at Onderstepoort and the Experiment Station at Dohne, Cape Province.

Selected farmers in each of the provinces of the Union were supplied with labelled tins in which they sent specimens of maggots collected from live sheep. The maggots, together with a small amount of wool, were posted to Onderstepoort from time to time beginning in September, 1940 and continuing until April, 1942. On arrival at the laboratory the maggots were placed on meat kept in insect boxes and reared to flies which were then identified and recorded. In most instances the larvae arrived in a healthy vigorous condition, but there were occasions when they arrived dead, largely as a result of packing too many in a tin and with too much damp and decomposing wool.

At Onderstepoort and Dohne maggots from individual sheep were collected and reared so that the records of infestations at these two places are more detailed than those obtained from collections made by farmers. In the latter collections maggots from more than one infested animal were mixed together in samples sent here for identification. In the circumstances it was impossible to get collections from individual sheep sent separately.

The surveys at Onderstepoort and Dohne were made by Mr. P. A. Cilliers from August, 1939 to August, 1940, and September, 1940 to April, 1941 respectively. The results of these two surveys and that from collections made by farmers are shown in the accompanying Table 1.

A glance at this table shows that of 324 collections of maggots, 179 or 55 per cent. were composed solely of *Lucilia cuprina*. In combination with other species this fly is responsible for about 90 per cent. of the total strikes. *Chrysomyia chloropyga* comes next in importance, while *Lucilia sericata* plays a minor rôle.

Unfortunately the collections sent in by farmers in Natal and the Transvaal were insufficient to enable valid comparisons with those from the other two provinces to be made. From the few records available it is interesting to note that the majority of strikes were made by *Lucilia cuprina* and *Chrysomyia chloropyga* in combination. In general, the rainfall and humidity of the sheepfarming areas in the Eastern Transvaal and Natal is higher than that of the sheep areas in the Cape and Orange Free State. Whether this factor could be correlated with a higher incidence of *Ch. chloropyga* strike is one for investigation. A project designed to collect data on this aspect of the blowfly problem was drawn up at the commencement of the investigation, and a few preliminary experiments were run, but owing to an increase of work in other phases this had to be abandoned. Briefly, the scheme planned was to expose individual sheep in fly-proof cages in which separate species of blowflies were liberated. Data on temperature and humidity and the numbers of strikes were to be recorded; records of the microclimate of struck areas of the animals were also to be obtained. Chemical treatment of the sheep was also contemplated. In this way it was hoped to gather information which might lead to a better understanding of the behaviour of the species of sheep blowflies.

TABLE 1.

Flies reared from Larvae collected from Struck Sheep.

	Total No. of Collections.	No. of Collections Comprising :— *L. cuprina.*	*L. sericata.*	*Ch. chloropyga.*	*Ch. albiceps.*	*L. cuprina* and *L. sericata.*	*L. cuprina* and *Ch. chloropyga.*	*L. sericata* and *Ch. chloropyga.*	*L. cuprina, L. sericata* and *Ch. chloropyga.*	*L. cuprina, Ch. chloropyga, L. sericata* and *Ch. albiceps.*	*L. cuprina, Ch. chloropyga,* and *Ch. albiceps.*
1. Collections by farmers—											
(*a*) Orange Free State	49	26	1	0	2	8	6	0	6	0	0
(*b*) Transvaal.......	12	2	0	3	0	0	7	0	0	0	0
(*c*) Natal...........	12	1	0	2	0	0	9	0	0	0	0
(*d*) Cape............	79	39	1	2	0	8	16	1	10	1	1
TOTAL......	152	68	2	7	2	16	38	1	16	1	1
2. Collections at Onderstepoort....	116	57	1	14	0	11	24	2	7	0	0
3. Collections made at Dohne......	56	54	0	0	0	0	2	0	0	0	0
GRAND TOTAL	324	179	3	21	2	27	64	3	23	1	1
Percentage of Total..	—	55	1	6	1	8	20	1	7	0·5	0·5

The Trapping of Blowflies.

Some remarks on this subject may not be out of place in this paper. In order to gain some idea of the prevalence of blowflies at Onderstepoort traps baited with meat were exposed almost continuously from May, 1940 to July, 1942. Fresh minced meat with water was put in a trap every week. At some times only one trap was run, but for the most part two traps were run concurrently, one containing fresh bait and the other bait which was seven days older. Catches were removed every three or four days and recorded. A graph (Graph I) was drawn to show the average mean daily catch per trap for each month and the average maximum and minimum temperatures were plotted. Rainfall figures were also indicated.

The catches of *Lucilia cuprina* began to increase steadily from July, 1940 to September, then dropped to twenty-three per trap per day in November, after that there was rapid rise reaching a peak (120) in December. From this time onwards a steady decline set in until March, when there was a minor peak (22), thereafter a rapid drop to one fly per day. Low catches continued throughout the winter of 1941, but an increase occurred in September and the highest peak was reached in February, 1942. This high point (34) was very much lower than that of the previous year. This may, perhaps be attributed to the summer being hotter and drier than the previous one.

The curves for *Chrysomyia chloropyga* indicate it to be most abundant in September and October of each year. Here again the catches for 1941 were lower than those for 1940.

Chrysomyia albiceps reaches its maximum peak in October to December with a minor one in May. *Chrysomyia marginalis*, from these trap records, appears to be most abundant from October to January or February. The maximum daily catch obtained with this species was five. In this connection it is interesting to note that there were occasions when fresh sheep carcasses attracted hundreds of these flies, while the bait traps about one hundred yards away were not catching any. Furthermore, attempts at rearing this species in cages proved very difficult as oviposition was most erratic. It would appear that stimuli required to induce oviposition by this species are not the same as those required by, say *Lucilia cuprina*. It is reasonable to argue that *Chrysomyia marginalis* may not be so readily attracted to the usual meat baits as *Lucilia cuprina*. It follows, therefore, that the fly catches in meat-bait traps may not give a true indication of the relative density of the population of the different species.

Lucilia cuprina is known to be attracted more readily to meat-bait in the early stages of decomposition than to baits in the more advanced stages of decomposition, whereas *Chrysomyia albiceps* is attracted more to the latter. For any given period of trapping, therefore, to obtain an idea of the density of population of the different species of flies, the baits most attractive to them should be exposed. The importance of *Ch. marginalis* will be discussed in paper No. V of this series. Inasmuch as it does not attack living sheep; but is a good scavenger, there is no object in trying to find a highly attractive bait for this species.

The correlation of strike incidence with fly catches in traps may perhaps be demonstrated sometimes: From records compiled by Mr. P. A. Cilliers at Dohne, for the period September, 1940 to April, 1941, fifty-six collections of maggots from individual sheep were made. Of these *Lucilia cuprina* was

responsible for fifty-four, while the two remaining ones comprised *L. cuprina* and *Chrysomyia chloropyga*. For the same period the numbers of flies taken in meat-bait traps were: *Lucilia cuprina* 4,420; *Lucilia sericata* 58; *Chrysomyia chloropyga* 4,265; *Chrysomyia albiceps* 1,570 and *Chrysomyia marginalis* 175.

Conclusions.

1. From collections of maggots from live sheep at Onderstepoort, Dohne, C.P. and the main sheep farming areas of the Union of South Africa, *Lucilia cuprina* alone was responsible for 55 per cent. of the total strikes, and in combination with other species the percentage was increased to 90.

2. *Chrysomyia chloropyga*, as a sheep myiasis producing fly, ranks next in importance to *Lucilia cuprina*.

3. From trapping records at Onderstepoort the seasonal abundance of blowflies are obtained. The necessity for using selective baits is stressed.

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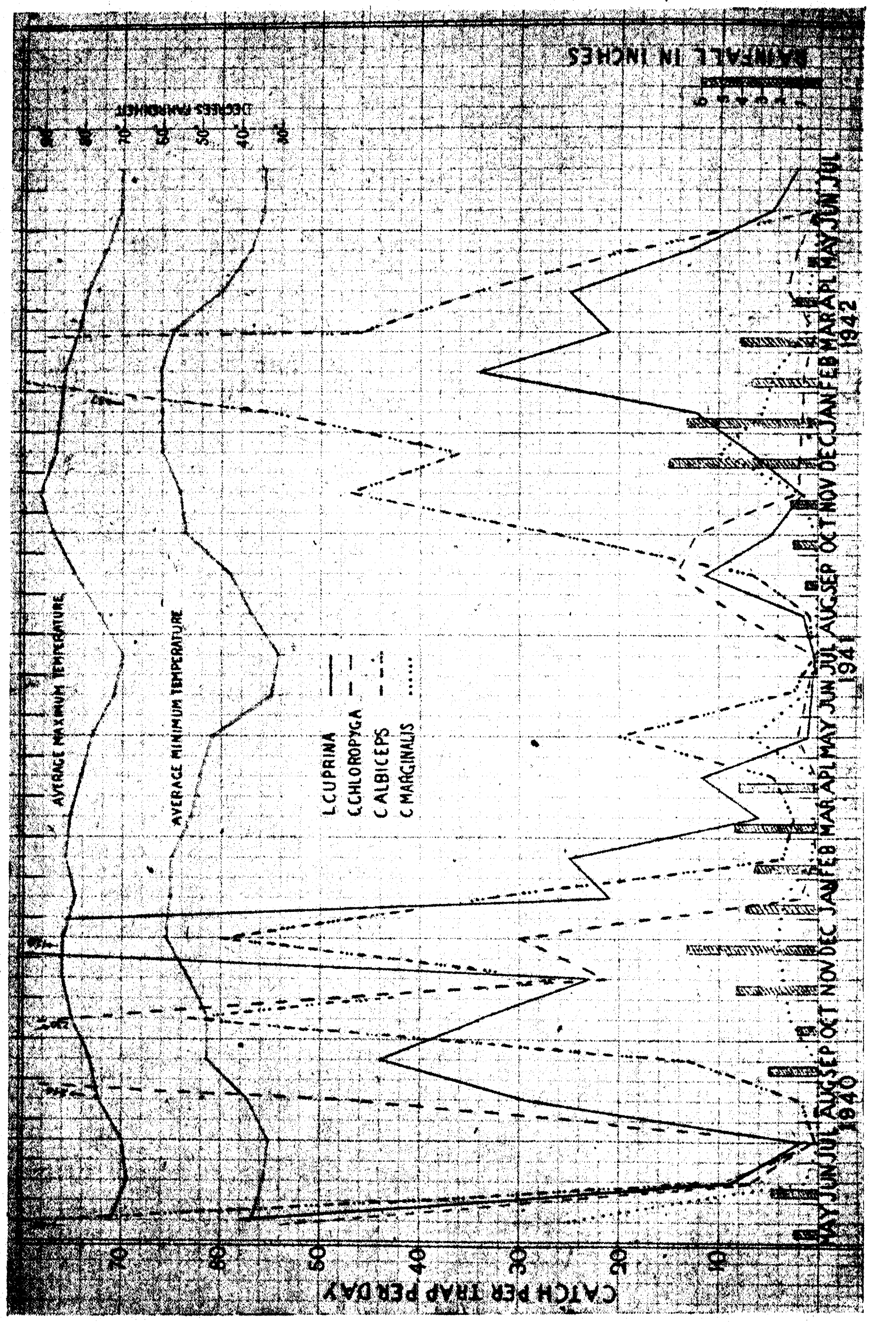
GRAPH I.


CATCH PER TRAP PER DAY
RAINFALL IN INCHES
DEGREES FAHRENHEIT
AVERAGE MAXIMUM TEMPERATURE
AVERAGE MINIMUM TEMPERATURE
L.CUPRINA
C.CHLOROPYGA
C.ALBICEPS
C.MARGINALIS
1940
1941
1942

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Sheep Blowfly Research II.—Suint Investigations.*

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Introduction.

According to the majority of the investigators, the olfactory oviposition stimulus supplied to the blowfly by the living sheep has its origin in the fleece of the sheep. Although it is realized that products of bacterial decomposition from moist parts of the skin probably are responsible for this stimulus, it has often been suggested that a correlation exists between amount and/or quality of wool suint and attractiveness to blowflies. Suint, being very hygroscopic, retains or attracts moisture, so providing the prerequisite for bacterial action in the epithelial débris and dried body exudates on the skin. Mönnig (1940) has given a concise description of the conditions that lead to blowfly strike, so that further details will be superfluous here.

Quantity of suint secreted in relation to blowfly strike has been the subject of studies by a number of research workers. The evidence has not been conclusive on the whole [*cf.* Holdaway and Mulhearn (1934) and Hobson 1936)]. Another variable that could conceivably operate in the problem of susceptibility is the composition of the suint itself, which may give rise to variable products of decomposition. These and similar considerations led to a tentative programme of suint analysis at this Institute in May, 1940, in conjunction with an extensive blowfly research programme. This suint investigation embraced two distinct projects, i.e. (1) basic work on suint in general, to discover which constituents of suint are attractive to blowflies, and (2) a qualitative and quantitative analysis of a series of fleece samples from selected examples of susceptible and non-susceptible sheep. The result of project No. (1) was to be used in No. (2), and the results of No. (2) could serve as a confirmation or otherwise of No. (1), while the information so obtained might be useful in the preparation of artificial baits for blowfiies.

Suint Constituents and Problems of Suint Analysis.

As regards the composition of wool suint, this complex problem has been submitted to systematic study by only a few investigators, notably Freney (1934). In a comprehensive survey Freney (1940) has reviewed present knowledge. Only a few salient points need, therefore, be mentioned here. It is known that the major portion of suint consists of potassium carbonate and organic compounds. The inorganic constituents have often been determined, and do not appear to vary outside certain narrow limits. This knowledge, naturally, does not enable one to indicate which inorganic compounds were excreted as such, but this is probably not of great consequence. A source of variation of inorganic constituents of wool suint that

* See footnote to article I of this series.

should always be kept in mind, is the nature of the soil of the sheep's environment. The fleece is always contaminated with dust, sand and vegetable material, which then contribute their part to the soluble alkaline solution of suint. The variation thus introduced into the chemical composition may even overshadow that due to variation between individuals.

Of the organic part of suint only a comparatively small percentage has been identified. The reason for this is not far to seek. Not only are we dealing here with a very complex mixture, the known constituents of which are mainly present in very low percentages, but probably this mixture exists in a kind of chemical equilibrium. Chemical manipulation would disturb this equilibrium, with a resultant formation or disappearance of some of the constituents, so confusing the analytical results. Furthermore, the suint solution has to be concentrated by boiling or by evaporation at an elevated temperature, a process that continues for hours and even days. This is a relatively drastic treatment where one is dealing with volatile substances present in very low concentrations. The probability that at this stage the suint is still identical in composition with that existing in the fleece, appears to be slight. In addition, it is quite possible that the water-soluble ingredients of fleece samples that are to be stored in not too dry an atmosphere will not remain constant over extended periods. The products of possible bacterial and oxidative changes may affect the problem one way or another [*cf.* Freney (1940)].

A further important consideration is that a large number of the known organic constituents of suint are homologous or otherwise inter-related compounds (e.g., the fatty acids), all of which together constitute only a small fraction of the whole mixture. Granted a successful isolation of this complex, further separation of it into its individual constituents is a most formidable task with the quantities likely to be available after isolation. If, further, one considers what this implies when analysing individual fleece samples (of about 10 to 15 grammes), for some definite suint constituent, the nature of the problem confronting one is perhaps better appreciated.

Investigation of Suint.

Freney (1937) found slight indications of attractiveness to *Calliphora augur* of butyric and valeric acids, whereas Parman *et al* (1927) had found n-valeric acid to be slightly attractive to a different blowfly the screw-wormfly *Cochliomyia macellaria*, but n-caproic and n-caprylic acids were repellent to this fly. Laake *et al* (1931), on the other hand, found butyric aldehyde to be slightly attractive to *Lucilia* spp. Freney (1937), again, found a mixture of sodium caproate and sodium sulphide to attract *Lucilia cuprina*, whereas sodium sulphide alone was unattractive. These results do not appear very convincing when the actual figures are examined, but they seemed to justify further investigation. If it could be shown that butyric, valeric and caproic acids are present in suint to any significant extent, a correlation between suint composition and blowfly strike may be shown to exist. With this end in view it was decided to concentrate initially on the study of the organic acids in the fleece.

At the outset attention was devoted to the different methods of direct extraction of raw wool. It was desirable that in the course of the extraction and concentration of the wash liquors, the suint constituents should be subjected to conditions as mild as possible. High temperatures for continued periods as well as a long series of manipulations and treatments

appeared undesirable, for reasons set out above in the section on problems of suint analysis. Any direct extraction of the constituents concerned was, therefore, to be preferred. Experimentation with various methods of wool extraction soon demonstrated the chief difficulty to be encountered, viz., the bulkiness of wool and its absorbing powers for solvents. These properties entailed special measures for an efficient removal of any residual solvent after washing, otherwise an important percentage of the original quantity would not be recovered, so wasting solvent as well as dissolved substances. With water as the extraction medium the first-named factor is of no account, but the last-named is a very important consideration. As large quantities of wool had to be extracted for the present purpose, the washing of small samples at a time was impracticable. The use of larger quantities of solvent, as in repeated washing or rinsing, was also inadvisable because of the difficulties of filtration and concentration. A technique that appeared to suit the purpose admirably was that developed by Mr. S. D. Rossouw, of the Wool Section of this Institute, a modification of the procedure described by Rossouw (1938). This consists of consecutive Soxhlet extractions of absolutely dry wool with petroleum ether, absolute alcohol and water, all under reduced pressure. Any moisture in the wool would interfere with the desired extraction by removing some of the water-soluble constituents on being syphoned over with the petroleum ether. Alternatively, in the extraction with absolute alcohol, moisture would carry into solution some substances otherwise insoluble in absolute alcohol. This would result in misleading extractions, so that working with absolutely dry materials is imperative. Wool and suint are both extremely hygroscopic, therefore it calls for careful manipulation and special facilities to achieve satisfactory results.

This procedure would bring about the extraction of all the potassium salts of organic acids in the fleece by the alcohol. The desired separation would thus be effected by comparatively mild and simple means. Preliminary extractions according to this technique showed, however, that extractions would have to be performed with large quantities of wool. The available apparatus did not allow of this; moreover, owing to war-time conditions, suitable apparatus could not be procured. The alternative course, viz., direct extraction by means of water alone, precludes most of these difficulties, and for the present purpose it did not appear that serious objections could be raised in this respect. Freney (1940), in his discussion of various methods of fleece analysis, concluded in a similar comparison of two methods resembling the two under discussion, that neither of the two could be wholly rejected in favour of the other. The circumstances, as well as the purpose for which the investigations are undertaken, must determine the procedure to be adopted. Once the choice has fallen on a suint extraction by means of water, it also entails the concentration of the relatively dilute suint solutions for analytical purposes.

The suint used in the majority of these experiments was derived from merino lox, a soiled type of wool containing a relatively high percentage of water-soluble substances. This wool was sampled at random from a number of merino fleeces. A few extractions were also made of the whole fleece of individual sheep, but this practice was ultimately discontinued.

In the course of the extraction of suint from the wool, a number of difficulties had to be overcome. To collect as much suint as possible it was desirable to scour the maximum amount of wool in a limited quantity of

water. The water was kept at 50° C. The scouring liquor was of a fairly "thick" consistency and offered considerable difficulty in filtration (a more dilute scouring liquor does not filter with any greater ease). The time required for filtration was sufficient to allow bacterial changes to occur in the liquor. As this bacterial action was also liable to affect the acidic components in the liquor, there was every reason for accelerating the process as far as possible. The filtrate had to be concentrated by evaporation either by boiling or by heating on a hot water bath kept at 70° C. This was a time-consuming process. On cooling, the concentrate was subjected to further treatment by one of the four methods described below.

The extraction by means of organic solvents was usually complicated by the formation of relatively stable emulsions. This was overcome by centrifuging, if possible, although the small capacity of the available centrifuge limited its use considerably.

The following methods of isolation of the organic acids were investigated.

1. That described by Rimington and Stewart (1932), where an ethyl alcohol solution of the acids liberated by acidification of the suint is salted out by means of ammonium sulphate.

2. That of Lassar-Cohn (1923), where an ethyl alcohol solution of the potassium salts of the organic acids is salted out with potassium carbonate.

3. Extraction of the acidified suint by means of solvents like ethyl ether.

4. Steam distillation of acidified suint for the recovery of the volatile acids in the distillate.

These different methods all yielded suprisingly small amounts of organic acids. In the case of numbers 1 and 2 the alcohol extracts naturally contained other organic material as well; in particular was that evident in Lassar-Cohn's method, where wax-like substances were soon precipitated on concentration of the original alcohol solution. In every case the amount of total acids derived from the extracts was equivalent to less than 20·ml. tenth-normal potassium hydroxide. The weight of raw wool concerned was roughly 500 grammes in each case. A few details concerning the results with each method follow below.

Method No. 1.

Although this method appeared to yield the best proportion of acids, these acids could not be shown to be mainly of low molecular weight. Even steam distillation of part of the extract did not yield volatile acids in any appreciable quantities, and this distillate still gave a precipitate with zinc acetate, indicating the presence of fatty acids higher than valeric acid. The major part of the acid complex appeared, from acid value determinations, to consist chiefly of oleic acid.

Method No. 2.

This method of extraction yielded an alcoholic solution of potassium salts of various organic acids. On evaporation of the alcohol the reddish-brown residue was once more taken up in absolute alcohol, but a considerable portion would not re-dissolve. The alcohol-soluble portion was again freed of alcohol, and the residue taken up in water. This solution gave a reddish-brown, gelatinous precipitate with barium nitrate solution; on filtering what

appeared to be a voluminous precipitate it was found that it amounted to hardly more than a trace. Acidification of this precipitate and subsequent extraction with ether gave an ultimate small residue having a strong, "rancid" odour.

A portion of the aqueous solution above was acidified with sulphuric acid, and shaken out, successively, with petroleum ether and ethyl ether. The residues from these last two extracts were weighed, and the acid values determined, viz., 126 for the petroleum ether and 183 for the ether extract. These values showed that we were dealing with mixtures of higher fatty acids with non-acid substances; there was no evidence of the presence of any of the lower fatty acids, oleic acid being probably the chief constituent. When these petroleum ether and ethyl ether extracts were neutralized with KOH, evaporated to dryness, washed with benzene and afterwards taken up in water acidified with sulphuric acid, steam distillation did not effect any separation of volatile fatty acids. Traces of a white, waxy solid floating on the distillate of the ethyl ether extract was identified as benzoic acid (cf. below). This acid was obviously derived from the hippuric acid known to be in suint. It is possible that traces of some of the intermediate fatty acids, e.g., capric and lauric, also occurred in the distillate, but that could not be demonstrated.

Method No. 3.

The extraction of acidified suint solutions with ethyl ether or petroleum ether was not accomplished without difficulty, the chief obstacle being the ease with which the liquids were emulsified. When dealing with dilute suint extracts these emulsions could be broken by appropriate manipulation, but stronger suint extracts defied any ordinary treatment. Ethyl ether was more prone to this tendency than petroleum ether, but, on the other hand, ethyl ether appeared to be much more desirable for the efficient extraction of lower fatty acids.

The extracts thus derived from suint did not provide sufficient material for further investigation. Accordingly, attention was turned to the possibility of obtaining more promising extracts by collecting bigger quantities of suint in an absolutely dry state. This was successively extracted with petroleum ether, absolute alcohol and water. These extracts were then worked up for acid constituents, but in no case could an appreciable amount of acids be separated. The present facilities for the rapid handling of large quantities of suint extracts (scouring liquors) were not available at the time, so that this method was eventually abandoned for the one about to be described.

Method No. 4.

It was hoped that by distillation a quicker separation, of the volatile acids at least, would be effected than by other methods. A large amount of very concentrated suint solution (rather more like a thin paste) was therefore steam-distilled after acidification with sulphuric acid. For various reasons a modification was soon introduced, i.e., the distillation was carried out at reduced pressure, so that it actually was not a steam distillation any more. This arrangement appeared more satisfactory, as the escaping gases or vapours were drawn through an alkaline solution to absorb any acidic vapours. A further modification of this technique was finally adopted when

raw wool was used directly for the distillation, instead of a suint extract. The wool was soaked thoroughly in water in a large distillation vessel, the pH was adjusted to about 3·0 by means of sulphuric acid, and distillation commenced. In this way the long and tedious process of suint extraction was eliminated.

The various distillates obtained by this method were tested for acidity. Some were extracted by means of ether, after an initial concentration by evaporation in alkaline medium. The odour of these distillates strongly reminded one of the smells of a sheep's kraal. The only acid, however, that was present in appreciable quantities, was a white waxy solid floating on the distillate. This acid appeared responsible for the peculiar odour of the distillates. Further purification, however, revealed it to be benzoic acid. No other acid could be identified in any of the distillates.

Some of the concentrates of the acids from suint extracts were tested by Mr. G. A. Hepburn in a special olfactometer (see paper No. 3 of this series), in order to determine the olfactory value for the sheep blowfly. The benzoic acid collected from the distillates above was also given a preliminary test in an olfactometer. This preparation was still in a crude state, with some of the "sheep" odour still adhering to it. In none of these cases was a promising indication found. The absence of any response on the part of the flies did not stimulate the belief in the presence of one or more attractive substances in sheep's suint.

Discussion and Conclusions.

The appearance of benzoic acid in the distillates of suint was confirmed by more than one method of separation. In every case the distillate was derived from a strongly acidified solution of suint products. Hippuric acid has been identified in suint by various workers, so that the appearance of benzoic acid in the distillate is probably the result of hydrolysis of hippuric acid in a boiling acid medium, with a subsequent volatilization in steam.

With regard to the olfactory tests carried out with some of these suint products, it should be borne in mind that there was a gradual evolution of the olfactometer as used at present (see contribution No. 3 of this series). Any tests undertaken, therefore, had to depend on the concurrently used apparatus. Thus it is possible that some of the results of tests with the earlier type of olfactometer are misleading. Re-testing of some of the suint products may, therefore, be desirable. Owing to the fact that the writer has also been implicated in other concurrent projects pertaining to the blowfly problem, it has not been possible to do any re-testing up to the present. With the sudden termination of this research programme it has now been found impossible to repeat this work.

It was stated in the introduction that fundamental work on suint would be followed up by a systematic analysis of individual wool samples from susceptible and non-susceptible sheep. This aspect of the investigation naturally depended on the first part. In collaboration with this Institute, Mr. A. H. de Vries, entomologist at the Grootfontein College of Agriculture, Middelburg, C.P., regularly collected a large number of fleece samples from specially selected groups of sheep, with this object in view, i.e., of investigating the problem of susceptibility in sheep in relation to the amount of suint, or to the amount of some attractive constituent of suint. As no advance was made with the search for an attractive suint ingredient, the

investigation of the fleece samples was delayed. At the termination of this work, therefore, nothing has been attempted in that respect. A quantitaive analysis of these fleece samples for total suint alone, may perhaps be profitable. The technique employed by Freney (1940) should be well suited to this purpose.

In conclusion the following may be said:—

1. Attempts have been made to isolate and estimate some of the organic acids, chiefly lower fatty acids, said to be present in small quantities in suint. Apart from the identification of benzoic acid as a decomposition product of hippuric acid, a satisfactory separation of these acids could not be achieved, owing to the small quantities present.

2. In olfactory tests, suint preparations and extracts (e.g. acid fractions) failed to attract blowflies. No correlation between suint composition and blowfly strike could, therefore, be demonstrated.

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Sheep Blowfly Research III.—Studies on the Olfactory Reactions of Sheep Blowflies.*

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The Blowfly Problem.

THE Joint Blowfly Committee of Australia in their report No. 2, 1940 (The Prevention and Treatment of Blowfly Strike in Sheep) deal comprehensively with this subject. They discuss sheep blowfly control measures under three groups:—

(*a*) measures to reduce inherent predisposition;
(*b*) measures to reduce immediate susceptibility;
(*c*) measures to reduce fly abundance.

In the third group would fall the trapping of flies, the disposal of carcasses as sources of blowflies and the treatment of strike. Experience in the Union of South Africa has shown that the most important breeding place of *L. cuprina* is the live sheep. This appears to be true under Australian conditions as well. In the control of sheep blowflies, therefore, efficient treatment of strike is of primary importance. This phase of the investigation is dealt with in article VI of this series.

An investigation on the part played by carcasses in the blowfly complex was undertaken at Onderstepoort, a report on it appearing in article V of this series.

I. M. Mackerras and Fuller (1936) have shown that extensive use of traps will reduce the incidence of fly strike by over 50 per cent. In these tests trapping was done on a very intensive scale. The average distribution of traps was one to about twenty-five acres. The bait used was meat. To bait on a comparable scale on most sheep farms in the Karroo would require eighty traps to a paddock one thousand morgen in extent. And when it is considered that many farms comprise five thousand morgen and more, the practical difficulties of maintaining such a large number of traps may readily be appreciated. The cost both in initial outlay and maintenance would be extremely great. Whether trapping on such a scale would be necessary to obtain similar results under our conditions is not known, but it is safe to say that, even if it could be reduced to 75 per cent., the costs would be too great for the average farmer.

On the other hand, if an efficient inexpensive bait could be found, trapping, combined with all the other suggested measures, might be considered an economic proposition.

* See footnote to article I of this series.

With the object of trying to find a bait which would meet all requirements, this phase of the investigation was commenced in April, 1940, by the writers, and carried on until September, 1942.

The properties of an ideal bait may be stated thus:

(*a*) It should be very attractive to *Lucilia cuprina*.

(*b*) It must be attractive for a reasonably long period, thus obviating its frequent renewal.

(*c*) It must be cheap.

(*d*) It must be readily procurable and easy to prepare.

Meat bait and in some cases meat treated with calcium or sodium sulphide is the only bait that has been used for trapping blowflies. It lures *L. cuprina*, but in addition it attracts, especially when old, *Ch. albiceps*, *Ch. chloropyga*, *Sarcophaga* spp. and *Musca* spp. It does not long remain attractive to *L. cuprina*, hence it requires renewal every week in summer: furthermore, it is not cheap. From the above requirements it will readily be conceded that meat does not make a satisfactory bait. However, of hundreds of substances tested by many workers in this field of research, nothing has been found to equal it in attractiveness to blowflies.

It was with some misgivings, therefore, that a search for a suitable bait was undertaken. In the following account the various aspects of the investigation are described. The investigation is very incomplete, but it is felt that although the results are largely negative in the broad sense, publication of the data may be useful to other investigators.

Freney (1937) suggested that studies on olfactory responses of blowflies should be conducted under laboratory controlled conditions rather than in the field, and the writers being of the same opinion, the major portion of this work was carried out in the laboratory.

To perform any work on olfactory responses of insects, suitable apparatus is a prime essential. Several different olfactometers have been devised for use with blowflies, but the ideal has not yet been attained. Dr. H. O. Mönnig of this Institution designed an olfactometer which was under construction prior to the commencement of this investigation and which was a modification of the olfactometer described by Lee (1937). It was completed shortly after commencement of these studies and the early work with blowflies was carried out by means of this apparatus. Although it was shown that this olfactometer could work, its great drawback was its unwieldiness. Much time was consumed in testing the apparatus and making alterations to its construction. Probably, after certain more adjustments are made, it will be found to give good service. But as the investigations proceeded it was felt that simpler apparatus of a type more easily handled was required. So, after many months, work with this instrument was stopped and attentions were transferred to another kind of apparatus. This apparatus, known as a cage-olfactometer, has been described elsewhere in this journal, Hepburn (1943). While this apparatus is not without certain disadvantages, its ability to indicate attractiveness or otherwise of baits in short exposures has been well demonstrated. It has the advantage too of being very simple and cheap to construct, so that ultimately four of these cages were made, thus allowing several experiments to be run concurrently.

These cage-olfactometers were set up in a small dark room which was kept at a constant temperature of 27° C. and a relative humidity of 40-50 per cent. Plans had been drawn up for air-conditioning this dark room, but as the investigation was rather suddenly terminated, these were not carried out. Although the room was not ventilated, no serious ill-effects on experiments were observed, though the desirability was recognized at the commencement of the investigation.

METHODS.

The blowflies were reared in an insectary and sufficient numbers were collected daily in cages and removed to an aquarium room, where they remained for one or two days before being used in experiments. While in the insectary and in the aquarium room, they were fed on sugar and raw meat, and given a liberal supply of water. Eighteen to twenty hours before they were used in an experiment their meat was removed from the cages. The temperature of the aquarium rom was maintained at 23-25° C., while the minimum relative humidity was 30 per cent. Flies ranging in age from three to fourteen days were used in the experiments, though at times, perhaps, older ones only were available. Under ideal arrangements it may be an advantage to work with flies of the same age, but this would necessitate a special organization for breeding flies in quantities sufficient for the purpose. Under present circumstances this was impossible and the writers had to accommodate themselves accordingly.

It would be desirable also for studies on chemotropic behaviour to be confined to one sex at a time, but it was not possible to do this. The separation of sufficient numbers of females and males for daily experiments in four olfactometers could have been achieved with an adequate staff. In these experiments, therefore, mixed sexes were used, but in the counts of trapped flies the sex was noted. Thus, any sexual response to an odour would have been detected.

METHOD OF TESTING.

For each experiment about one hundred flies are used. The baits to be tested are put into small Petri-dishes, screened, and placed within glass traps. In some cases the test substance is run *versus* water, a blank, or an attractive beef bait. Care must be taken where test substances are in solution to compare equal volumes of them and their controls, and to have their surface areas of exposure the same. Attractiveness of a substance depends on many factors, but that of concentration is very important, especially in experiments where two odours are being compared.

The time required for each experiment depends upon the activity of the flies, and the olfactory quality of the test substance. In general it was found that exposures of one to two hours were long enough for the flies to react. At the end of each exposure the apertures in the cages are closed and the two traps removed for the counting and sexing of the trapped flies. While this is being done in another room, the lights in the dark room are switched off and the flies left to rest until the experiment is continued. The flies in the traps are anaesthetised with ether or chloroform, removed, sexed and counted. The glass traps, wire cones and screens are then ventilated in bright sunlight, and the Petri-dishes are cleaned out and fresh test substances put in, prior to continuing the experiment. When the experiment is continued, the test substances are replaced in their respective traps,

but these are now reversed in position, the former left hand one being placed on the right hand side and *vice versa.* When a reaction has taken place the experiment is ended and the flies removed for counting and sexing in the manner just indicated. The figures obtained give four totals, one for the test substance on the left side, one for that on the right side, and the two catches of the control, left and right. The relative attractiveness of the test substances to that of its control can be calculated by using the formula given in a publication by Ripley and Hepburn (1929a). The reason for reversing the baited traps is to eliminate, as far as possible, errors due to position factors.

The procedure adopted in this investigation was to run tests in the following manner. Water-soluble substances were tested against water to ascertain whether they were attractive or otherwise. A substance catching consistently more than water was then tested against a standard bait described on page 31. If the substance caught fewer flies than water it was regarded as non-attractive or even repellent. Substances which, when tested against blanks, caught less than the latter, were regarded as repellents. To test obscurant properties, each trap was baited with a standard attractant and the test substance added in a separate container to one trap. A comparison of the relative catches gives a measure of the obscurant value of the substance.

Experiments which yielded doubtful or conflicting results were repeated until enough data were accumulated to permit conclusions to be drawn.

The Search for Attractants.

In conducting an organized search for substances which will attract blowflies, several lines of approach may be adopted. Freney (1937) outlines a scheme which gave valuable results. He implied that his investigation could have been carried out to more advantage perhaps, had it been done under laboratory controlled conditions. His scheme was largely used here as a basis for these tests on olfactory responses of sheep blowflies.

Apart from testing substances which are known or assumed to occur in decomposing protein, it is not unreasonable to carry the search among substances which are not found in the environment of the flies. Working with fruitflies, Ripley and Hepburn (1931) found certain attractive substances, e.g., terpinyl acetate, which is not regarded as being associated with odours likely to occur in their natural environment. Similarly for blowflies, it would be wise to test as many substances as possible in the hope that a suitable attractant may be discovered. This, of course, may entail years of laborious research with perhaps disappointing results.

In our scheme it was planned to test as many substances as possible, but under present world conditions the difficulties of obtaining them are almost insuperable. Some of the chemicals which Freney listed as of great promise as attractants were obtained, and others which were considered might be suitable, were ordered. Some of these were tested, but the majority were unobtainable. Results obtained with pure chemicals will be given later and discussed (page 37).

As *L. cuprina* is greatly attracted to damp wool in the breech of certain sheep, special study was given to substances occurring in suint and this will be dealt with on page 32. A chemical analysis of suint was undertaken and reported on in article II of this series.

Particularly in the drier parts of the Union of South Africa there occur certain interesting xerophytic plants of the genus *Stapelia*, which produce remarkable flowers emitting an odour strongly resembling that of decomposing carrion. Blowflies are greatly attracted to these flowers and they freely oviposit on them. It was felt that if the attractive substances produced in these flowers could be isolated and identified, a valuable clue would be obtained to the solution of the problem of successful blowfly attractants. This phase of the investigation will be discussed on page 42.

The treatment of meat with various chemicals also received considerable attention. Attempts to make extracts of attractive beef baits were made using different solvents, e.g., petroleum-ether, ethyl ether, ethyl acetate, ethyl alcohol, maize oil and liquid paraffin (i.e., medicinal paraffin). It was hoped that well-defined differences in attractiveness of these extracts might be shown. Having thus found the most suitable solvent, further chemical treatment should eventually lead to the identification of some of the active constituents of the bait.

Finally, some attention was devoted to testing fermenting mixtures of fish meal, pancreatin and eggs; gelatin and blood serum; and hydrolysed casein. In some instances the bait was inoculated with mixed cultures of bacteria obtained originally from the intestinal flora of sheep.

A chronological survey of the work is not favoured, for, as the investigation proceeded, recapitulations of experiments were necessary and new ideas were obtained. Details of technique too, were improved, as the studies were furthered.

In the beginning it was considered desirable to have a standard attractant with which to compare test substances. No pure chemical having the requirements was known. Ethyl mercaptan and bromoform were found by other workers to attract blowflies to field traps. At the time the former could not be procured and the latter was found to be unattractive when used in dark room tests. The writers, therefore, were forced to use decomposing meat as the bait. In the beginning minced bovine liver and water were used, but later a change was made to minced beef. It was found that the bovine livers were very variable in quality. The reasons for this were numerous, but one of the main causes was due to their storage in refrigeration for varying periods. A fresh liver allowed to decompose in water appeared to produce different odours from that of a liver, say, kept previously for weeks in cold storage. As the supply of beef was more constant, it was decided to make the change. An attractive bait was made by mixing equal quantities of minced beef and water and allowing this to decompose for several days before use in the olfactory tests. But even in this great variation was encountered, and it was suspected that meat from different parts of the same carcass would produce differences in attractiveness. The work was often handicapped by the bait turning sour, or by contamination by moulds and fungi. Finally, although not entirely satisfactory, meat bait was prepared thus: Beef was obtained from cold storage and then minced; equal quantities of this and water were placed in a Mason jar, inoculated with a mixed bacterial culture and incubated at 37° C. for 40 hours. The bait container was kept closed, but not sealed. The fermenting mixture was then centrifuged at 3,000 r.p.m. for about twenty minutes and the liquid portion thus separated was decanted and transferred to stoppered

bottles. This portion was used as the "standard attractant". In many experiments this "beef soup" was diluted instead of being used in concentrated form.

The attractiveness of beef bait depends, apart from the other factors, on its age. In the preparation of our "standard attractant" it was found that it was more attractive after being incubated for 48 hours than for 24 hours, although the latter was also attractive. Incubation for 72 hours caused a reduction in attractiveness. The attractiveness of the bait was enhanced by the inoculation of bacteria, and, judged by human senses, it appeared to produce more penetrating odours than the non-inoculated one.

Tests with Suint Preparations.

The suint extracts described in contribution no. II of this series was subjected to a number of olfactory tests during the course of this investigation. With the Mönnig olfactometer, in which the first tests were run, results obtained with steam distillates of acidified suint concentrates were disappointing, although there was just an indication of slight attractiveness. These distillates had all been neutralized with potassium hydroxide after distillation. A slight excess of the alkali was added and the solution evaporated to a small volume before olfactory tests were commenced. In the light of later results obtained with potassium and sodium hydroxides, an indication of attractiveness with these distillates may be misleading. Any such indication of attractiveness may not be due so much to the distillate than to the alkali present in excess. Further tests in this older type of olfactometer could not throw any more light on this problem. The crude benzoic acid derived from the steam distillate of acidified raw wool appeared to be very slightly attractive. The weak response by the flies and variation in individual trap catches point to its doubtful olfactory value. Further repetitions of tests with suint distillates were even more unsatisfactory. It was not clear whether the poor results were due to the condition of the blowflies, the quality of the distillates or to defects in the apparatus.

When the cage-olfactometers were used, a few further tests with suint products were made. A highly concentrated suint extract (a filtered and concentrated water extract of lox) was not attractive when tested against water or beef bait. Similar results were obtained with a concentrated solution of the potassium salts of the steam distilled acids of acidified suint solution, again in the presence of slight excess of potassium hydroxide.

Further tests were made with two extracts derived thus: the steam distillate of acidified merino lox was made slightly alkaline with potassium hydroxide, concentrated by evaporation, acidified with sulphuric acid, and extracted in a separating funnel by means of either petroleum ether or ethyl ether. These two extracts were allowed to evaporate spontaneously after which they were tested in the cage-olfactometer. The three species of blowflies *L. cuprina*, *L. sericata* and *Ch. chloropyga* were used together in these tests, the results of which were negative.

Experiments with Meat Bait to which Chemicals had been Added.

In the first experiments bovine liver baits were used to which were added calcium carbonate, calcium sulphide and sodium sulphide at the rate of 3 per cent. of the total weight of meat and water, while in the later

work done with the cage-olfactometer, we had changed over to a beef medium. Here the range of substances added to meat was extended and the following were tested: cystine, phenothiazine, sodium thiosulphate, and sodium carbonate.

An interesting result was obtained by comparing liver and water and the same plus cystine at 0·75 per cent. of the total weight. Both baits were incubated at 37° C. for fourteen days. Firstly, the control was run against water and found to be five times as attractive (*L. cuprina* was used in the test). Then the untreated and cystine treated baits were compared, four grams of each being used. Two complete experiments, each with reversals, were carried out, the cystine bait being superior, trapping 171 flies and the control 116. 70 per cent. of the former were females and 59 per cent. of the latter. Of the total number of flies used in the experiment (466) 62·8 per cent. were females.

In another experiment using beef and beef plus cystine at 0·66 per cent. concentration, both ten days old at room temperature, the latter was twice as attractive as the former to *L. cuprina* but equal in attractiveness to *Ch. chloropyga*. No significant difference in sexual attractiveness was demonstrated. In other tests using the latest technique, i.e., incubation at 37° C. and centrifuging, the addition of 1 per cent. cystine to beef enhanced its attractiveness. The experiment was run in triplicate and in each case the percentage of trapped females was higher in the treated than in the untreated bait. In this experiment the baits were incubated for nineteen hours. In further tests using baits incubated for forty hours the superior attractiveness of cystine-beef was again demonstrated in four replicates, but no significant difference in sexual attractiveness was shown. The addition of cystine to a meat bait would facilitate the evolution of sulphur compounds, e.g., mercaptans and sulphides (see Wohlgemuth, 1904), and these may account for the increase in attractiveness of this bait to *L. cuprina*. Furthermore, such chemicals may be more attractive to females but, owing to the greater volatility of some portion, much of this attractiveness in a nineteen hours incubated bait is lost on further incubation.

A comparison of beef bait inoculated with bacteria and beef bait plus 1 per cent. cystine, both incubated for forty-five hours, showed that the latter caught more flies than the former (nearly twice as many) but, during the first fifteen minutes of the experiment more flies were attracted to the inoculated beef than to the cystine-beef which finally showed superiority. Similar results were obtained from other experiments. The addition of cystine 0·67 per cent. to a beef bait containing 2·66 per cent. calcium carbonate in one instance and 0·8 per cent. sodium carbonate in another, failed to increase attractiveness to *L. cuprina* or *Ch. chloropyga*. In the former the two baits attracted *L. cuprina* equally, but the calcium carbonate meat bait was more attractive to *Ch. chloropyga* than the cystine. The sodium carbonate meat bait appeared to be slightly more attractive to *L. cuprina* and more markedly so to *Ch. chloropyga* than the cystine bait.

Calcium carbonate: The addition of calcium carbonate at a concentration of 2·7 per cent. of the total weight of the bait increases attractiveness. A comparative test with treated and untreated baits, five days old, shows that the treated is more attractive to *L. cuprina* and *Ch. chloropyga*, while for the latter species there is an indication that females are more attracted than males. Later tests with sixteen and thirty days old bait show that the

treated bait is more than twice as attractive to *L. cuprina* and about four times as attractive to *Ch. chloropyga*. In other words, the addition of this substance at first enhances attractiveness and then maintains it more effectively than does an untreated bait.

Sodium carbonate: Meat bait containing 0·8 per cent. of sodium carbonate was tested against meat and calcium carbonate 2·7 per cent., both baits being six days old. They were equal in attractiveness to *L. cuprina* and *Ch. chloropyga*.

Sodium sulphide: The results obtained with this substance in the cage-olfactometer were not highly satisfactory. In the early stages of this investigation tests indicated that meat and sodium sulphide 3 per cent. was more attractive than meat plus calcium sulphide or meat plus calcium carbonate. The results were variable and no final conclusions on the effect of sodium sulphide could be drawn. Further experimentation is thus indicated.

Phenothiazine: At a concentration of 1·33 per cent. this compound raised the attractiveness of beef both to *L. cuprina* and *Ch. chloropyga*. A mixture of phenothiazine and sodium carbonate in meat is also attractive, but it is not clear to which substance this attractiveness is mainly to be attributed.

Sodium thiosulphate: 1 per cent. of this salt in a thirteen days old fish-meal bait increased its attractiveness for a day, but thereafter it apparently lost its effect.

When testing the above substances in meat baits the writers were handicapped by having only one cage-olfactometer. In one case seven meat baits with chemicals added and an untreated control were prepared together. It was impossible to test these baits individually at the same ages, but it was endeavoured to obtain as many different tests as possible. This work has been left incomplete but enough data were obtained to show the general effects produced by such chemical treatment of meat.

Field Experiments.

Although the difficulties surrounding trapping experiments under field conditions were fully appreciated, some tests were carried out with chemically treated meat baits.

In these experiments the baits were exposed in large oil drum traps which were so constructed as to prevent visiting or trapped flies from ovipositing on the bait. In the first experiment eight traps were used. Four baits, each one being duplicated, were set out in a circle in a paddock adjacent to sheep pens. The baits were alternated in position and after each recording of trapped flies each bait was moved in position to the adjacent trap, working in a clockwise manner. By this method errors due to position factor were somewhat reduced.

Eight kilograms of fresh minced bovine liver was equally divided into eight portions and to each was added one litre of water. Two of these were left untreated as controls while the remainder were each chemically treated. The chemicals used were calcium carbonate, calcium sulphide, and sodium sulphide, each at a concentration of 3 per cent. of the total weight of the bait plus water. The experiment was commenced on the 22nd April, 1941, and continued until May 26th.

Unfortunately there was not an abundance of flies, and despite the changing of bait positions there were big individual variations. After the first day the two control baits did not catch. Calcium carbonate bait attracted more blowflies than the other two treated baits, especially *Ch. albiceps*, a secondary fly. Calcium sulphide was the least attractive of the treated baits. As far as *L. cuprina* was concerned the calcium carbonate bait was somewhat more attractive than the sodium sulphide one.

TABLE 1.

Species of Blowfly.	Number of Flies trapped by baits.			
	Control.	Sodium Sulphide.	Calcium Sulphide.	Calcium Carbonate.
L. cuprina	1	64	47	106
L. sericata	1	4	2	92
Ch. chloropyga	2	81	9	208
Ch. albiceps	5	335	34	2,683
Ch. marginalis	15	713	217	602
TOTAL	24	1,199	309	3,691

Calcium carbonate meat bait trapped nearly as many *L. sericata* as *L. cuprina*, while it was also attractive to *Ch. chloropyga*, a not unimportant primary fly. The results are shown in Table 1. With these field experiments one of the most disconcerting factors is that of variation. This may be offset very largely perhaps by having four or five replicates of each bait per experiment.

From the experiments listed it appears that the addition of unstable sulphur compounds to meat or carrion baits produces substances which increase the attractiveness. A critical survey of the results from all these different chemical treatments of meat and carrion failed to indicate any specific compound responsible for this attractiveness.

In addition to these various treatments of meat bait, experiments with small carcasses chemically treated were run under field conditions. It was thought at the time that results obtained by the addition of chemicals to minced meat and water might not be parallel to those obtained by similar chemical treatment of whole carcasses. Furthermore, from a practical viewpoint any chemical treatment of carcasses to render them more attractive, and, at the same time toxic to flies, should be investigated. With these objects in view some preliminary tests were made. It has been found more convenient to discuss them and also the subject of hydrogen ion concentration of meat and carrion baits chemically treated, in the following paper, No. IV of this series.

FERMENTING BAITS.

Apart from the systematic method adopted in the search for attractants, the writers experimented at random with various fermenting mixtures in the hope of meeting something of a promising nature. Some years ago Dr. M. Sterne, one of the bacteriologists at this Institution, noted that while

he was working with some accidentally contaminated *botulinus* cultures, offensive odours were given off which apparently attracted swarms of flies to his laboratory. Unfortunately the species of flies invading the laboratory were not identified and the nuisance created by these insects was so great that the bacterial cultures were destroyed. Subsequently, on request, attempts by this bacteriologist were made to rediscover these contaminative organisms, but with no success. Many cultures were made and exposed to the different species of blowflies, and while some were weakly attractive the majority appeared not to be greatly promising. It is intersting to note, however, that one of these cultures when put in a cage containing *L. cuprina*, immediately stimulated a few flies to copulate. Further investigations on cultures of this type might lead to the discovery of a valuable sexual attractant to blowflies. This phase of the investigation had to be abandoned because there was no bacteriologist available to continue the work.

The following baits all inoculated with bacteria were tested under field conditions: unsterilized meat plus unboiled culture media, unsterilized meat plus boiled culture media, sterile meat plus boiled culture media, and sterile meat plus unboiled media. These were supplied by Dr. E. M. Robinson of Onderstepoort. They were exposed simultaneously in ordinary field traps, the first two for five days and the latter two for seven days. The first-mentioned was the most attractive, catching 34 *L. cuprina*, 200 *L. sericata*, 360 *Ch. chloropyga*, 53 *Ch. albiceps* and 53 *Ch. marginalis*. The attractiveness of the baits lasted only for the few days they were exposed.

In another experiment the following baits were exposed in traps for fifteen days during November, 1940: minced bovine liver and water, 6 *L. sericata;* minced liver and water plus bacteria from sheep intestines, 5 *L. cuprina*, 25 *L. sericata*, 2 *Ch. albiceps*, 30 *Sarcophaga*, 180 *Musca spp.*; blood meal and water, 16 *L. sericata*, 2 *Ch. albiceps* and approximately 700 *Musca* spp.; blood meal and water inoculated with bateria, 2 *L. cuprina*, 10 *L. sericata*, 1 *Ch. chloropyga*, 1 *Ch. albiceps*, 40 *Sarcophaga* and approximately 700 *Musca* spp.; fish meal and water, 56 *L. cuprina*, 56 *L. sericata*, 72 *Ch. albiceps*, 50 *Sarcophaga* and over 3,000 *Musca* spp.; fish meal and water, inoculated, gave no catch. The fish meal bait was promising and further tests were made later.

Laboratory Experiments.

Fish meal baits.—Variable results were obtained with these baits. The following mixtures were tested: Fish meal 50 gm., pancreatin 0·5 gm., 1 egg, and water 200 c.c., and the same plus 0·5 gm. arsenic trisulphide. A comparison of these two at three days old and then at five days, showed the former to be more attractive, but it was not so attractive as a beef bait twenty days old. However, when the former fish mixture was six days old it was apparently more attractive than beef bait eight days and twenty-three days old. A fish meal bait of the same formula but without the addition of an egg was found to be attractive when ten days old and superior to an eleven days old beef bait. This bait was kept in a closed jar in a room at 25° C. When repeated later the bait was kept in an open jar and, compared with similarly treated beef baits, it failed to attract so well as beef, when two and fifteen days old. Finally, another mixture including an egg, was found when twelve days old, to become more attractive than a five day old beef bait. Further work in this direction is indicated in order to answer the question concerning fish meal as a suitable medium for an attractive bait.

The following preparations were also tested: (a) gelatin 12·5 gm., egg yolk 42·5 gm., egg albumen 50 gm., cystine 2·5 gm. and water 200 c.c.; (b) blood serum-gelatin media. Both these baits were made in duplicate, one of each being inoculated with bacterial cultures. The blood serum mixture with and without bacteria gave unpromising results, but the egg gelatin and cystine bait, inoculated and non-inoculated, was appreciably attractive, but not better than beef baits. Freney obtained promising results with casein hydrolysed with sodium sulphide, but the writers did not: 50 gm. of casein were mixed in 200 c.c. of water containing five gm. of sodium sulphide, and kept in a stoppered jar at room temperature. Tests on the third and sixth days showed that no attractive products had been formed.

Field Experiment.

Finally, a field test with addled eggs was run. Small holes were drilled through the egg shells and a water infusion of horse manure inoculated into each egg. The holes were then sealed by means of wax. After incubating these eggs at 26° C. for seven days they were beaten up in a dish and put in a field trap. A similar lot of eggs incubated for nine days was also placed in a field trap. Both lots attracted all the species of blowflies. The peak of attractiveness was reached about three days after the traps were put out and flies were still attracted on the fourteenth day, but not in large numbers.

Tests with Pure Chemicals.

All the experiments with pure chemicals were carried out in the laboratory under conditions described elsewhere in this report. The intention was to test many of the chemicals known to be products of proteolytic decomposition, but unfortunately only some of those ordered could be procured. All the tests made are listed in Table 2.

The tests were qualitative, designed to discover attractants. With the exception of two or three of the chemicals all were tested in the cage-olfactometer. An important consideration in olfactometer tests is the dilution of the test substance. It is conceivable that a chemical may be attractive at one concentration and not attractive or even repellent at another. A further factor to consider is that of the solvent for the test substance. In this investigation most of the substances used were soluble in water, but some had to be dissolved in oil or alcohol. In choosing oil solvents care must be taken to use one which is olfactorily neutral to the flies. Maize oil and "liquid paraffin" meet this requirement. The use of alcohol as a solvent is not entirely satisfactory unless it can be used very dilute. In concentrations of more than 10 per cent. it should not be used for the vapour given off stuns and even kills the flies approaching the traps. On the other hand, solutions of the test substance in weak alcohol often are too easily thrown into suspension, with a portion of the suspended particles floating on the liquid which virtually amounts to the chemical being exposed undiluted on the surface. The solvents and the dilutions at which the tests were made have been specified in our list, for it is our opinion that the publication of data without this information would lose much of its value to other workers engaged in the same or similar lines of research.

TABLE 2.

Chemicals Tested in Cage-olfactometers.

Chemical.	Dilution.	Solvent.	Control.	Olfactory Result.
Bromoform	Very weak	Water	Beef bait	Not attractive.
Bromoform	Very weak	Water	Water	Not attractive.
Ethyl alcohol	10 per cent	Water	Water	Repellent.
Linalool	10 per cent	Maize oil	Maize oil	Repellent.
Valeric aldehyde	20 drops	50 ml. of water	Water	Weak attractant.
Sulphaldehyde	0·03 per cent	Water	Water	Weak repellent.
Sulphaldehyde	0·03 per cent	Water	Beef bait	Not attractive.
Carvone	5 per cent	Maize oil	Maize oil	Repellent.
Carvone	10 per cent	Maize oil	Maize oil	Repellent.
Carvone plus beef bait	5 per cent	Maize oil	Beef bait	Obscurant.
Musk ketone	Pure	—	Blank	Weak attract. to *L. cuprina*. No reaction with *Ch. chloropyga*.
Phenyl acetic acid	0·8 per cent	Water	Water	Indefinite.
Phenyl acetic acid	0·8 per cent	Water	Beef bait	Not attractive.
n—Butyric acid	2 per cent	Water	Water	Not attractive.
n—Valeric acid	0·1 per cent	Water	Water	No reaction.
n—Valeric acid	0·1 per cent	Water	Beef bait	Not attractive.
iso—Valeric acid	0·1 per cent	Water	Water	Neutral.
iso—Valeric acid	0·1 per cent	Water	Water	Not attractive to *L. cuprina*, *L. sericata* or *Ch. chloropyga*.
n—Caproic acid	0·2 per cent	Water	Water	Not attractive.
n—Caproic acid	0·1 per cent	Water	Water	Repellent.
n—Caproic acid	0·2 per cent	Water	Beef bait	Not attractive.
n—Caproic acid plus Sodium sulphide	0·8 per cent and 1 per cent	Water	Sodium sulphide 1 per cent. in water	Equally attractive.
n—Caprylic acid	·02 ml. in 100 ml. of water; solution drawn off at bottom	—	Water	Neutral.
Erucic acid	Pure	—	Blank	Indefinite to both *L. cuprina* and *Ch. chloropyga*.
Thioacetic acid	0·1 per cent	Water	Water	Neutral to *L. cuprina*, *L. sericata* and *Ch. chloropyga*.
Thiopropionic acid	0·05 per cent	Water	Water	Conflicting, probably not attractive to *L. cuprina* and *Ch. chloropyga*.
Thiobutyric acid	1 per cent	Water	Water	Not attractive.
Thiobutyric acid	0·1 per cent	Water	Water	Not attractive.
Thiobutyric acid	0·01 per cent	Water	Water	Not attractive.
Thiobutyric acid	0·11 per cent	Ethyl alcohol, 10 per cent. in water	Ethyl alcohol, 10 per cent. in water	Slightly attractive.

TABLE 2.—(*continued*).

Chemical.	Dilution.	Solvent.	Control.	Olfactory Result.
Thiobutyric acid plus beef bait	0·11 per cent	Ethyl alcohol, 10 per cent. in water	Ethyl alcohol, 10 per cent. in water (plus beef)	Obscurant.
Thiobutyric acid	0·11 per cent	Ethyl alcohol, 10 per cent. in water	Beef bait	Not attractive.
Ammonium salt of n—Butyric acid	1·7 per cent	Water	Water	Slightly repellent to *L. cuprina*, *L. sericata* and *Ch. chloropyga*.
Ammonium salt of iso-valeric acid	1·7 per cent	Water	Water	Slightly repellent to *L. cuprina*, *L. sericata* and *Ch. chloropyga*.
Ethyl caprylate	4 drops on water	—	Water	Probably repellent.
Ethyl caprylate	4 drops on water	—	Beef bait	Not attractive.
Amyl caprylate	4 drops on water	—	Beef bait	Not attractive.
Amyl caprylate	4 drops on water	—	Water	Not attractive.
Linalyl acetate	10 per cent	—	Maize oil	Neutral.
Linalyl acetate	Pure	—	Blank	Slightly repellent.
Phenyl glycol diacetate	5 drops on water	—	Water	Neutral.
Phenyl glycol diacetate	5 drops on water	—	Beef bait	Not attractive.
Ethyl mercaptan	1 : 50,000	Water	Water	Not attractive.
	1 : 100,000	Water	Water plus beef bait	Not attractive.
	1 : 200,000	Water	Water	Not attractive.
	1 : 500,000	Water	Water	Not attractive.
Ethyl mercaptan and Indole	1 : 200,000 and 0·05 per cent	Water	Water	Not attractive.
Ethyl sulphide	0·125 per cent	Water	Ethyl sulphide 0·05 per cent.	More attractive than control.
	0·05 per cent	Water	Water	Not attractive.
	0·05 per cent	Water	Beef bait	Not attractive.
	0·1 per cent	Water	Water	Not attractive.
	0·25 per cent	Water	Water	Not attractive.
	1 : 100,000	Water	Water	Neutral.
	1 : 100,000	Water	Beef bait	Not attractive.
	0·04 per cent	*Liq. paraf	*Liq. paraf	Neutral.
	0·04 per cent	*Liq. paraf	Beef bait	Not attractive.
Ethyl disulphide	0·025 per cent	Water	—	Not attractive.
	0·1 per cent	*Liq. paraf	—	Weak attractant.
Ethyl disulphide plus a dish of water	0·1 per cent	*Liq. paraf	Beef bait	Not attractive.
Triethyl amine	1 per cent	Water	Water	Weak repellent to *Ch. chloropyga*, *L. cuprina*.
	1 per cent. exposed in sun 40 minutes	Water	Water	No reaction from *Ch. chloropyga*, *L. cuprina*.
	0·25 per cent	Water	Water	No reaction from *Ch. chloropyga*, *L. cuprina*

* Medicinal paraffin.

TABLE 2.—(*continued*).

Chemical.	Dilution.	Solvent.	Control.	Olfactory Result.
Butyronitrile	0·5 per cent	Water	Water	Not attractive to *L. cuprina and Ch. chloropyga.*
Butyronitrile	0·5 per cent	Water	Beef bait	Not attractive to *Ch. chloropyga and L. cuprina.*
Indole	0·05 per cent	Water	—	Weak attractant.
Indole	0·05 per cent	Water plus 5 per cent alcohol	—	Indefinite.
Indole	1 per cent	Water plus 40 per cent. alcohol	—	Flies stupefied.
Carbazole	Powder floating on water	—	Water	Neutral to *L. cuprina, L. sericata, Ch. chloropyga.*
Maize oil	Undiluted	—	*Liq. paraf	Both neutral.
Natural civet	Undiluted	—	Blank	Slightly attractive.
Dippel's oil	Undiluted	—	Blank	Strong repellent.
Dippel's oil	1 per cent	Maize oil	Maize oil	Repellent.
Potassium hydroxide	24 per cent	Water	Water	Attractive.
Potassium hydroxide	0·72 per cent	Water	Water	Attractive.
Potassium hydroxide	0·72 per cent	Water	Beef bait	Not attractive.
Potassium hydroxide, boiled solution, high grade quality	20 per cent	Water	Potassium hydroxide 5 per cent. boiled solution, high grade	Equal in attractiveness.
Potassium hydroxide, purest grade, boiled solution	20 per cent	Water	Potassium hydroxide high grade, 20 per cent. boiled solution	Both attractive, little difference between them.
Sodium hydroxide	20 per cent	Water	Water	Attractive.
Sodium hydroxide	20 per cent	Water	Beef bait	Slightly attractive.
Sodium hydroxide	20 per cent	Boiled water solution	Water	Attractive.
Potassium carbonate	20 per cent	Water	Water	Slightly attractive.
Potassium carbonate	20 per cent	Water	Beef bait	Not attractive.
Calcium hydroxide	Saturated water solution	—	Water	Slightly attractive.
Sodium thiosulphate	20 per cent	Water	Water	Slightly repellent.
Sodium bicarbonate	9 per cent	Water	Water	Repellent.
Sodium sulphide	1 per cent	Water	Water	Slightly attractive.
Sodium sulphide and Ammonium carbonate	1·9 per cent and 2·2 per cent	Water	Ammonium carbonate 2·2 per cent. in water	Not attractive.

Ethyl mercaptan and other organic sulphur compounds found to be promising by Freney were given extensive tests. The former was tested at concentrations in water varying from 1:50,000 to 1:500,000 and also in combination with indole, with negative results. A 0·05 per cent. solution of indole was only slightly attractive. 1 per cent. sodium sulphide in water was slightly attractive and a mixture of sodium sulphide 1 per cent. and caproic acid 0·8 per cent. was slightly attractive, but caproic acid 1 per cent. alone was somewhat repellent. It seems likely, therefore, that the attractiveness of the mixture may be due to the sulphide. Thiobutyric acid 0·11 per cent. in 10 per cent. ethyl alcohol is slightly attractive, but when tested against a beef bait this attractiveness is not demonstrated. Concentrated valeric aldehyde is slightly attractive to *L. cuprina*. Undiluted natural civet and musk ketone* are also slightly attractive. Of repellent substances found carvone, Dippel's oil and linalool were the most outstanding.

It will be shown later in the discussion on experiments with various chemically treated extracts that certain apparently non-volatile substances, e.g., potassium hydroxide, were found to be attractive to *L. cuprina*. Even boiling the solution was found to reduce its attractiveness only to a small degree. Minute traces of volatile impurities must apparently be responsible for this attraction for the reaction was olfactory and not a visual one. That the blowfly is remarkably sensitive to certain odours is well-known; the results quoted above with tests on potassium hydroxide indicate a very delicate olfactory mechanism of the fly. In the course of the experiments in which meat bait controls were used, attempts were made to determine the threshold of attractiveness with beef baits. Beef bait was prepared in the manner already described and tests were run with this at various dilutions. Five drops of "beef soup", i.e., 0·13 c.c. diluted in 2·37 c.c. water were found to attract *L. cuprina* readily, while a solution twice as strong showed no greater attractiveness. A solution five times the strength of that of the first-mentioned was appreciably more attractive and the optimum concentration was that of the undiluted "soup". In all these tests the volume of bait used in each trap was 2·5 c.c. For most tests a 50 per cent. solution of soup and water was found to be satisfactory. Any chemical showing consistent superiority to such a control would be marked down for future field experiments.

It is interesting to recall Freney's experience with ethyl mercaptan which, he said, attracted many *L. cuprina* to the vicinity of field traps but did not induce them to enter. He put forward the theory that perhaps one set of odours attracts flies from a distance while another lures them into the traps.† In the tests with ethyl mercaptan at dilutions ranging from 1:50,000 to 1:500,000 no response was shown by the flies. This might be due to a matter of dilution or one of olfactory quality. It was hoped to test methyl mercaptan but this was unobtainable. It may be that a mixture of mercaptans or organic sulphides with chemicals like indole or skatole may be found to be attractive. The possibility of a single chemical attracting

* Musk ketone is a dinitro derivative of tert. butyl acetophenone or a similar ketone.

† Hobson (1938), working with *L. sericata*, states that the oviposition response consists of two phases: (1) attraction from a distance, and (2) stimulation to oviposit. Perhaps the attraction to ethyl mercaptan as observed by Freney falls within the scope of the former and does not supply a stimulus for oviposition.

the flies has not been ruled out but, from our work and judging by that of other workers, it is our opinion that a mixture of odoriferous substances is responsible for this attractiveness.

Experiments with Stapelia Flowers.

Mention has been made on page 31 of the attractiveness to blowflies of *Stapelia* flowers. There are hundreds of species of this genus and a comparative study would probably reveal specific differences of attractiveness to blowflies. It was found that one species, *Stapelia flavirostris*, was very attractive to *L. cuprina*. A day-old bloom was placed inside a large cage (2 by 2 by 2 feet) containing several hundred flies and immediately the insects swarmed over the flower to such an extent that no portion of it was visible. Subsequently, in cage-olfactometer tests, a flower attracted more flies than two cubic centimeters of an attractive bait.

It was felt that in this plant there was something which might well repay a chemical investigation. Accordingly, collections of *S. flavirostris* were made at Grootfontein College of Agriculture in the Karroo by Mr. George Gill, botanist, and Mr. Sutton, Government Veterinary Officer. These flowers were preserved in a weak solution of mercuric chloride and despatched to Onderstepoort.

We are indebted to Dr. H. L. de Waal, formerly of this Institution, for undertaking the chemical study of these flowers. He prepared steam distillates of the coronas and corollas, and also made extracts with various organic solvents in an effort to discover the most suitable method of extraction. At the time we did not have the cage-olfactometers, so the tests were carried out in the Mönnig olfactometer. All of the distillates were attractive to *L. cuprina*—even after prolonged distillation odoriferous material was carried over into the distillate. Some of the odours issuing from the receiver could be further absorbed in ice-cold water. Acidification with tartaric acid does not accelerate the distillation of the active ingredients, and addition of potassium hydroxide to the distilling flask does not appear to have any effect. Various organic solvents were also used for extraction. Chloroform seemed promising in the beginning but subsequently failed, as did all other solvents. Even a six-days extraction with ethyl ether in a bubble-extractor failed to remove any attractive ingredient from an attractive steam distillate of *Stapelia* coronas. Unfortunately there was insufficient material available for further work.

Several hundred plants were planted at Onderstepoort but these did not flower well the following season. From the blooms available from this source it was shown that (*a*) there is great variation in attractiveness of individual blooms, (*b*) attractiveness is correlated with the age of the blooms, e.g., a bloom picked when one to two days old is more attractive than a freshly opened one, (*c*) attractiveness disappears quickly after the bloom begins to wither.

The flowering period for this species is not long so that it is probable that further investigations on this problem will have to be continued for several seasons.

Experiments with Extracts of Beef Baits.

Extraction of beef baits was attempted only towards the close of this investigation, the sudden termination of which prevented the following up

of any promising indications. With the exception of a beef bait containing 1 per cent. cystine, all the extracts were made from standard beef bait inoculated with bacteria (*vide* page 31).

Preliminary tests indicated that the liquor or "soup" obtained from the bait by centrifuging at 3,000 r.p.m. contained as much as, if not more, of the active olfactory constituents than the solid proteinaceous material. It was decided, therefore, to concentrate on the bait liquor for the preparation of extracts.

Further tests showed that, on addition of an excess of 96 per cent. ethyl alcohol to the liquor, most of the active ingredients probably remained in solution. Repeated washing of the precipitate, i.e., the alcohol-insoluble protein in the liquor, apparently removed all attractive substances present. The residue of the alcohol-soluble portion after evaporation, on the other hand, proved only weakly attractive.

Trials with medicinal paraffin and maize oil as extractants proved disappointing when compared with other solvents. The original idea in using these non-volatile oils was to find a solvent which would be olfactorily neutral and serve as a satisfactory diluent, while the viscosity and vapour pressure of the solvent should be such as to allow only a slow volatilization of the odoriferous material dissolved in it. This principle has been recognised by Ripley and Hepburn (1931).

In this way it was hoped to prepare an extract from which the odour would emanate more slowly and uniformly than it would from ether extract residues. That would have been a useful advance, a step nearer the achievement of a "standard attractant". Judging by the human sense, odoriferous material had decidedly been extracted from the beef "soup" by these two oils, but the flies did not respond to them. It may be that these oils fixed the odours too successfully.

A further trial was made to fix the odours of beef soup in an emulsion with lanoline. The object was to dilute the beef soup in a medium which would permit only a slow evolution of odours, thus stabilizing the solution or mixture to a standard rate of production of odours. This is an extension of the principle of Ripley and Hepburn mentioned above.

Ethyl ether was ultimately selected as the most suitable solvent after some preliminary trials had shown it to be promising. The ease with which it emulsified with beef soup was an obstacle which was best overcome by centrifuging for short periods. In cold weather this did not entail a big loss of solvent by evaporation. For testing purposes, measured volumes of ether extract were evaporated spontaneously from weighing bottles which could be tightly stoppered with ground glass lids. It appeared that heating of the ether extract for rapid evaporation resulted in a big loss of olfactory material; evaporation before a fan did not appear to affect it so adversely so that this procedure was finally adopted. The bottle was stoppered immediately the last traces of ether were on the point of disappearing. Only when the test in the cage-olfactometer was about to commence was the bottle unstoppered and both bottle and lid were placed in the glass trap. A good quality ether was used in these extractions; those brands leaving pungent acidic residues were unsuitable.

The results consistently showed that ether removes some constituents from the beef soup that are very attractive to *L. cuprina*. It was also found that washing of the ether extract with water before evaporating it will

reduce its attractiveness but only to a slight extent. Further work was then undertaken to try if possible to isolate and identify some of the constituents present in the extract. Preliminary trials with the extraction of acidified or alkaline beef liquor were abandoned when the tendency to emulsification was found to have been enhanced. Instead, the ether extract itself was washed with sulphuric acid (12 per cent.) or with potassium hydroxide (20 per cent.) solutions. The residues of these washed extracts as well as the acid and alkaline washings were then tested in the cage-olfactometer. The results indicated that the acid did not reduce the attractiveness of the extract to any significant extent, whereas washing with the hydroxide reduced attractiveness appreciably. The acid washings of the extracts showed no signs of attractiveness; the alkaline washings, however, were attractive although acidification of these (with sulphuric acid) apparently destroyed every trace of attractiveness. This unexpected result was confusing and led to the olfactory testing of the reagents, i.e., sulphuric acid and potassium hydroxide.

Sulphuric acid proved to be unattractive but potassium hydroxide, of which a high grade product had been employed in all the tests, was found to be attractive and quite consistently so. Further tests with the highest grade of potassium hydroxide, i.e., the analytical reagent grade, confirmed these results. There seemed to be a slight difference in degree of attractiveness between the purest and the high grades of hydroxide, the latter being slightly more attractive. When the solutions of these two grades of potassium hydroxide had been boiled rapidly for periods varying from thirty minutes to an hour no significant reduction in attractiveness could be demonstrated. Should any volatile impurities (?), therefore, be responsible for this attractiveness, they must be of a moderately volatile nature and not be distilled off by ordinary boiling. Potassium hydroxide solutions of different concentrations did not show appreciable differences in attractiveness. Whereas 24, 20 and 5 per cent. solutions were about equally attractive, i.e.; caught on the average three to five times as many flies as the water control, a 0·72 per cent. solution was found to catch twice as many flies as the control.

Having thus found potassium hydroxide to be attractive to *L. cuprina* it was only natural to test sodium hydroxide. The analytical reagent grade was employed, and it showed the same degree of attractiveness as the potassium compound. Continued boiling also failed to destroy the attractiveness. The only treatment which appeared to destroy this property was one of acidification with sulphuric acid. This would lead one to suppose that the pH has some bearing on the matter.

A few tests with potassium carbonate and calcium hydroxide showed them to be slightly attractive: *L. cuprina* reacted positively to these alkalis only very slowly. On the introduction of potassium hydroxide to the experiment with potassium carbonate the flies reacted well in a very short time to the hydroxide solution and few only were caught in the trap containing the carbonate. Further, some tentative experiments were run with a 9 per cent. solution of sodium bicarbonate in duplicate tests, the results of which were very consistent, showing this solution to be unattractive, water catching four times as many flies. The flies were not thirsty for they were given an abundant supply of water throughout and before the running of the experiment.

The significance of pH as a factor in attractiveness is not clear from the results obtained with these solutions for, except in the case of sodium bicarbonate, the pH falls within the range of about 12·3 to 13·4.

At this stage the investigation came to a sudden termination so that a number of ideas could not be followed further. The available evidence does not suffice to allow of any generalization. It does not appear likely that the potassium or sodium ions in solution are responsible for attractiveness, especially in view of the negative result obtained with sodium bicarbonate. On the other hand, according to orthodox ideas, the hydroxyl ions can hardly provide the attractive agents. In any case, these inorganic compounds are not supposed to be volatile, so that one is inclined to the view that this strange phenomenon must be attributed to volatile impurities. This, however, appears to be difficult to reconcile with the fact that a 0·72 per cent. solution of potassium hydroxide also showed definite signs of attractiveness. In this experiment the absolute amount of potassium hydroxide employed per trap was only 36 milligrams. An impurity could only have been present in traces in the original preparation (KOH), so that in the test extremely minute amounts only could have been involved.

Discussion.

In our introductory remarks reference was made to the experiments of I. M. Mackerras and others on the effect of intensive trapping on the incidence of strike, and the economic obstacles to any practical application of the method were stressed. Ordinary meat bait and also meat bait plus calcium sulphide was used, both of which caught great numbers of flies. Mary Fuller (1934) demonstrated by field tests the great attractiveness of meat bait treated with sodium sulphide. It has been shown that the addition of cystine to meat baits increases their attractiveness. From these and many other observations by various investigators it seems reasonable to associate attractiveness of baits to blowflies with the production of sulphur compounds.

No simple and cheap substances have yet been found to attract blowflies as do meat baits or chemically treated meat. Fermenting egg mixtures also supply attractive odours to blowflies but it is doubtful whether baits like these could compete with meat baits. Fish meal bait was found to produce attractive odours, but results showed it to be rather disappointing in general. Furthermore, an expensive chemical, pancreatin, was required to make this bait attractive. All these substances, while attracting blowflies in great numbers, fail to attract sufficient numbers of *L. cuprina*, the most important of sheep blowflies, to reduce the incidence of strike.

Although hundreds of pure chemicals and scores of chemically treated substances have been examined for possible use against blowflies, no suitable attractant has been found. Further studies in this field may eventually lead to the discovery of an ideal bait. It must be added, however, that the discovery of an ideal bait will not solve the blowfly problem, but it will provide a valuable control measure. In a search for the ideal bait investigators must be prepared to explore, perhaps for a very long time, a wide range of substances. It may not be out of place to offer some suggestions for future work in the light of our experiences.

Hobson (1938) has shown from field observations that attraction is twofold; one factor is supplied by the living sheep and the others by products of putrefaction. He points out that tests with repellents should be done on living sheep and stresses the importance of using gravid females.

From our results oviposition has been shown also to be stimulated by the odour emanating from *Stapelia* flowers. No sheep factor is present in this instance. The desirability of continuing chemical studies on these flowers has also been mentioned.

The advantages which may result from using the sexes separately in chemotropic tests have also been mentioned on page 29. In the use of mixed sexes in olfactometer tests it is possible that a female in a highly attractive condition to males might enter a trap and attract flies, thus giving a result which might easily be very misleading.

Further work on extracts of attractive meat baits may lead to an elucidation of the nature of the attractive substances, while more tests with inorganic alkalis should also be carried out.

Our studies on suint preparations are also incomplete. The chemical difficulties in work of this nature are very formidable and have been mentioned in contribution No. II of this series.

As regards suint as a factor in susceptibility, the results obtained do not permit of any generalization, except that thus far no indication have been found of the presence of any attractive constituent in suint. This does not exclude the possibilty that suint may provide a source for products of decomposition that are attractive. The idea that acids like caproic and caprylic may be specially attractive has not been substantiated either by tests with these acids by themselves, or with extracts of suint calculated to have retained (isolated) these acids. These extracts were tested in the acid state, or when made alkaline with potassium hydroxide, and in neither case was there any positive response from the blowflies. The remaining possibility is that the amount of suint secreted may be of significance in susceptibility. This question has received some attention in the past, but does not seem to have been conclusively disposed of [cf. Hobson (1936b) and Holdaway and Mulhearn (1934)]. No further evidence can be adduced at this stage.

The use of repellents on sheep to prevent oviposition does not, according to Hobson (1940), offer much encouragement. The requirements for a satisfactory repellent are so rigid that it is doubtful if any could be found. So far no repellents have been found which will keep off flies from sheep for more than a week. The most promising results so far appear to be obtained with the blowfly spray for the treatment of myiasis (see article VI of this series).

Although several pure chemicals, e.g., sodium and potassium hydroxide, have been shown here to be attractive under certain conditions, and other workers have mentioned the effects of bromoform and ethyl mercaptan, nothing superior to meat bait has been found. It has been demonstrated how meat bait could be improved by the addition of cystine and by inoculation with bacterial cultures. In both these instances the attractive odours produced are probably very mixed, and in the latter they were found to be very volatile. It is our opinion, and this seems to be shared by many other investigators that, while it is not impossible for a single chemical compound to supply the necessary stimulus to the flies, attractiveness is to be found in a mixture of odours.

Summary.

1. In a search for olfactory attractants to sheep blowflies, tests were conducted by means of an olfactometer in the laboratory, while some substances were tested under field conditions in traps.

2. Certain alcohols, aliphatic acids, esters, organic sulphides and inorganic salts were tested in the laboratory; boiled and unboiled solutions of sodium and potassium hydroxide were found to attract *L. cuprina*, while natural civet and musk ketone were weakly attractive. Sodium bicarbonate solution was repellent. Strong repellents are Dippel's oil, carvone, and linalool.

3. Preparations of suint were found in general to be unattractive.

4. The chemical treatment of meat bait by the addition of cystine, calcium carbonate, calcium sulphide, sodium carbonate, sodium sulphide and phenothiazine, enhanced its attractiveness.

5. Inoculation of meat bait with a mixed culture of bacteria from sheep's intestines increases attractiveness.

6. Fermenting baits, e.g., fish meal, pancreatin and egg, and addled eggs, proved to be attractive, but they were not so attractive as meat baits.

7. Some of the attractive substances of beef bait were extracted by ethyl ether, but these chemicals were not isolated or identified. A portion of these attractive substances were apparently removed from the ether solution by potassium hydroxide solution.

8. Flowers of *Stapelia flavirostris* are strongly attractive to, and stimulate oviposition by *Lucilia cuprina*. Distillates of these flowers were found to be attractive but no chemicals were isolated or identified. Further investigations on the chemistry of these flowers are recommended.

9. No blowfly attractant superior to chemically treated beef bait has been found.

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Sheep Blowfly Research IV.—Field Tests with Chemically Treated Carcasses.*

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THE experiments described in this paper form part of a project which was planned to be continued later. After carrying out the following preliminary tests certain weaknesses in the method were revealed, but at the same time some facts, worthy of notice, emerged.

OBJECTS.

(*a*) To regulate the pH, if possible, of carcasses at a stage attractive to primary flies.

(*a*) To try to find a poison which would meet the above requirement and render the carcasses toxic to visiting flies and their progeny.

An account of experiments on the addition of buffering agents and other substances to minced meat bait has been given in paper III of this series, but a full discussion of the rôle played by pH was deferred to this paper for consideration. In addition the question of pH of chemically treated whole carcasses will be discussed.

METHODS.

As guinea-pig carcasses which were very uniform in size and convenient to handle in traps could readily be obtained, they were used in the tests on the chemical treatment of carrion. The fresh carcasses were opened from the oesophagus to the anus, and the test substances were dusted over the internal organs, exposed meat surfaces, tissues and the natural orifices. The pH, taken in a thigh muscle each time, was measured before, and immediately after the chemical was applied, thereafter at frequent intervals.

With the exception of experiment 6 the treated carcasses were placed in large oil drum traps. These traps are made from forty-four gallon oil drums with removable lids and bottoms. The metal lid of each has been almost completely cut out and a circular piece of cello-glass inserted to provide light for the flies. The blowflies enter the traps through eight wire mesh cones which have been inserted equidistantly around the wall of the drum. These cones are situated about fifteen inches above the base of the drum. The bait, kept in a suitable container, is placed inside the drum and

* See footnote to article I of this series.

rests on a metal lid fitted to the base. Above the bait container and a few inches below the cones a circular wire-mesh screen is attached to the inside of the drum. This prevents the trapped flies from having access to the bait and, to protect the bait from eggs or maggots falling through, a circular piece of stout paper slightly greater in area than that of the bait container, is fastened to the wire-mesh screen. The trapped flies are removed after being killed by spraying the interiors of the drums with pyrethrum oil spray.

Each treatment, as far as possible, was made in duplicate. The traps were set out in a rough circle in a paddock and they were alternated in position so that no two carcasses with the same chemical treatment were adjacent. Only in the last experiment in which four small traps were used were the traps changed in position daily. The results are shown in the following tables, Nos. 1 to 6.

The untreated carcasses (see Table 1) attracted more *L. cuprina* than the treated ones though no significant differences in total catches between the talc treatment and controls are shown. The experiment was designed to test the effect of dusting a carcass with an inert powder. Flies appear to come more readily in the first two days to untreated carcasses than to those with talc, but after that the latter seem to be as attractive.

The reasons for the low catch of the calcium carbonate may be due largely to position factors. (In this experiment the baits were not changed in position during the period of exposure.)

The pH of the thigh muscle was measured each time catches were recorded. No significant differences in values were noted and no correlation of pH with catch can be seen.

In the second experiment (see Table 2) the poisons were mixed with talc, one to three parts respectively. The amount of poison used was 0·25 per cent. of the weight of the carcass. The control carcass attracted relatively more flies of each species than the poisoned carcasses. The carcasses treated with sodium fluoride attracted fewer *L. cuprina* than the others but the data are insufficient to warrant the deduction of general conclusions.

In the third experiment ants interfered with the calcium chloracetate bait traps. The indication is that the untreated carcass attracts more flies, except perhaps *L. sericata*, than the chemically treated ones. (See Table 3.)

A comparison of the results of the fourth experiment (see Table 4) shows that sodium thiosulphate increases the catch of all species, but more particularly for *Ch. albiceps* and *Ch. marginalis*. Whereas borax shows little difference from that of the control for *Lucilia* species, it catches very few of the other species, viz., *Ch. chloropyga*, *Ch. albiceps* and *Ch. marginalis*.

In experiment five (see Table 5) the chemicals were used at a higher concentration than in the preceding ones. The carcasses treated with barium carbonate caught appreciably fewer flies than the other baits. A comparison of the results shown in Tables 4 and 5 shows that with an increase in concentration of sodium thiosulphate, the relative catches of *Lucilia* spp. by untreated and treated baits is not appreciably altered. There is actually a decrease in the total catch of *L. cuprina*, but the results show that the sodium thiosulphate-treated bait catches *L. cuprina* when the control has ceased to do so, and were the experiment prolonged, this treated bait might be found to be attractive for a longer period.

Table 4.

		Total Flies Trapped.	Untreated Carcass (ii).							Total Flies Trapped.	Sodium Borate, 1 Per Cent. (i).							Total Flies Trapped.	Sodium Borate, 1 Per Cent. (ii).							Total Flies Trapped.	
8	10	—	1	2	3	4	7	8	10	—	1	2	3	4	7	8	10	—	1	2	3	4	7	8	10	—	1
—	9	41	1	4	5	8	5	—	—	23	1	4	6	7	4	1	1	24	5	18	3	5	1	1	3	36	1
—	—	25	4	—	2	—	1	—	—	7	—	—	—	—	—	—	—	—	20	5	5	—	—	—	—	30	1
—	—	6	—	—	3	2	2	—	—	7	—	—	—	1	—	—	—	1	—	—	—	1	—	—	—	1	—
1	29	167	18	21	57	34	46	—	1	177	—	2	10	4	11	—	—	27	4	15	16	8	11	2	2	58	15
—	1	10	1	1	3	3	1	—	—	9	—	—	2	—	—	—	—	2	1	1	—	—	—	—	—	2	6
62	67	7,176	200	577	1,200	1,240	1,300	88	62	4,667	264	278	1,043	1,100	1,800	264	164	4,913	69	114	475	530	1,800	117	259	3,314	284
6	3	51	7	21	21	7	9	1	3	69	1	2	6	7	1	—	1	18	3	6	5	6	5	8	15	48	4
7·6	7·8	—	7·1	8·0	7·8	7·8	7·6	7·5	7·4	—	8·0	7·0	7·7	8·0	7·8	7·8	7·5	—	7·8	7·0	7·7	7·8	7·8	7·9	7·8	—	6·5
—	—	—	7·3	—	—	—	—	—	—	—	8·4	—	—	—	—	—	—	—	8·4	—	—	—	—	—	—	—	7·6

Table 5.

	Untreated Guinea-pig Carcass (i).					Total Flies Trapped.	Untreated Carcass (ii).					Total Flies Trapped.	Sodium Thiosulphate, 2·5 Per Cent. of Body Weight (i).					Total Flies Trapped.	Sodium Thiosulphate, 2·5 Per Cent. of Body Weight (ii).					Total Flies Trapped.	Barium 3 Per Body W	
....	2	4	6	8	11	—	2	4	6	8	11	—	2	4	6	8	11	—	2	4	6	8	11	—	2	4
....	18	19	3	4	3	47	8	6	4	—	—	18	4	7	6	7	8	32	11	4	0	2	8	25	3	4
....	28	7	7	3	0	45	58	16	1	1	—	76	37	40	20	7	8	112	33	56	6	7	[illegible]	106	5	2
....	—	2	1	1	—	4	—	1	—	—	—	1	—	6	6	2	2	16	—	—	1	1	—	2	—	—
....	37	105	29	20	49	240	35	64	15	1	—	115	8	64	168	115	116	561	1	36	12	19	73	141	22	34
....	8	8	1	—	—	17	27	—	—	—	—	27	1	5	15	18	4	43	—	2	—	—	—	2	—	—
....	720	1,280	800	456	148	3,404	400	1,600	400	67	23	2,490	143	600	1,000	1,440	1,360	4,543	58	136	220	340	200	954	52	200
....	16	35	22	10	6	89	20	46	13	10	—	89	9	35	34	44	53	175	6	30	33	31	40	140	8	33
....	7·7	7·9	8·0	8·0	8·0	—	7·6	8·0	7·8	7·9	7·8	—	7·3	7·9	7·9	7·8	7·6	—	7·7	7·8	7·9	7·8	7·4	—	7·7	8·0
....	7·1	—	—	—	—	—	7·4	—	—	—	—	—	6·8	—	—	—	—	—	6·8	—	—	—	—	—	7·6	—

Table 6.

ment.	Untreated Guinea-pig Carcass.				Total Flies Trapped.	Sodium Arsenate (0·5 Per Cent. of Carcass Weight), in Kaolin.								Total Flies Trapped.	Sodium Arsenate, 0·5 Per Cent. of Weight of Carcass, plus Calcium Carbonate 3 Per Cent.									Total Flies Trapped.	
....	1	2	3	4	—	1	2	3	4	7	10	11	15	—	1	2	3	4	7	8	10	11	15	—	1
....	1	29	34	2	66	—	1	—	—	—	—	—	—	1	1	—	—	—	1	—	—	—	—	2	—
....	31	98	68	2	199	—	2	—	1	—	—	—	—	3	3	—	—	1	2	—	—	1	—	7	3
....	70	31	296	28	425	—	—	—	—	—	—	—	—	—	—	—	—	—	1	2	1	—	—	4	1
....	17	164	520	169	870	—	—	—	—	—	—	—	—	—	—	—	—	—	1	5	6	1	—	13	3
....	27	19	34	2	82	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
....	25	224	1,360	480	2,089	—	12	—	—	4	—	—	—	16	2	—	—	—	40	2	1	—	—	45	7
....	12	9	14	6	41	—	10	1	—	6	5	—	—	22	5	—	2	1	18	26	18	5	3	78	3
....	7·4	7·6	?	?	—	7·6	6·5	6·8	7·0	7·8	7·9	7·9	7·8	—	7·0	6·5	6·5	7·0	7·5	7·7	7·8	7·9	8·0	—	7·9

In experiment six (see Table 6) the carcasses were exposed in "Improved" blowfly traps. The carcasses were put in porcelain dishes containing sand placed inside the lower portions or bait containers of the traps. Wire cones were fitted to the entrance holes of the traps to prevent the escape of visiting flies. The majority of the flies attracted to the traps passed from the bait container to the upper screened portion of the apparatus. The flies had access to the carcasses on which they deposited their eggs.

The effect of sodium arsenite on carcasses is well known. It renders them unattractive to flies causing them to mummify. The effect of the less commonly used insecticide, sodium arsenate, was not known. It was hoped that at fairly low concentrations (0·5 per cent.) the carcasses would develop highly attractive odours to blowflies and yet be sufficiently poisonous to them and their progeny.

Here, calcium arsenate, sodium arsenate plus kaolin, and sodium arsenate plus calcium carbonate were tested, together with an untreated control. Toxicity records of this experiment show that only a few larvae of *Sarcophaga* spp. survived; many eggs of blowflies were deposited on the carcasses, but in the treated carcasses larvae died in the first or second instars while very few reached the third instar. Many flies were reared from eggs deposited on the untreated carcass.

As far as attractiveness to flies is concerned it is clearly demonstrated that the treated carcasses are not attractive. The control carcass was highly attractive for four days after which it was destroyed by maggots.

The carcass containing calcium arsenate appears to be slightly more attractive than those containing sodium arsenate. We ker concentrations of these poisons may, perhaps, be found not to exert such an inhibitory effect on attractiveness of carrion.

A study of the pH measurements given in each table reveals no correlation with the numbers of flies trapped.

It is possible that the change in pH of decomposing meat may be related to its variation in attractiveness to primary blowflies. If that is so, and if it could be shown at what particular pH maximum attractiveness is attained, an interesting avenue is opened for exploration. With this idea in mind pH measurements were made on meat baits and on the guinea-pig carcasses described above, as well as on the sheep carcasses of the experiments described in contribution No. V of this series. An attempt at correlation of a general trend of pH change in decomposing meat with its change in attractiveness could be made by means of laboratory tests in the olfactometer, by regular catches in traps, and by observation on the carcasses. As no standardized meat bait could be evolved for cage-olfactometer tests, only the last-mentioned two methods were used. It was soon found that there is a general trend of pH change, but there is also considerable variation (except in the case of carcasses) within wide limits. There appears to be a bigger variation in pH in different localities of a carcass than between different carcasses. This confuses the issue, especially as pH measurements (carried out by means of a set of standardized indicator papers) in the same locality, appear to vary little during the period the carcass remains particularly attractive to primary blowflies. The length of this period varies considerably, depending chiefly on the average air temperature prevailing at the time. In hot summer weather the carcass would probably still attract primary flies after two or three days were it not for the activities of the

maggots developing in large numbers within the carcass. They quickly push up the pH of the tissue in their vicinity to 8·0, when the strongly ammoniacal vapour produced by the action of the maggots appears to deter the flies. Results of catches in traps and in the cage-olfactometer show that meat baits [consisting of meat and water (50:50)] of pH values varying between 5·6 and 8·1 may be attractive to primary flies. Below a pH of 5·6 meat baits were usually found unattractive. The variable catches did not allow of an assessment of the degree of attractiveness at various pH-levels. The pH of a bait, therefore, is not an adequate measure of the bait's potentialities.

In view of these findings attention was turned to another aspect of the problem, i.e., buffering of meat baits. It was hoped that a few effective buffers for meat would be discovered, covering a fair range of pH. Olfactory tests with these buffered baits would then show at what pH optimum results are to be expected. Accordingly, a few chemicals were selected as possible buffers, viz., borax, disodium phosphate, calcium acetate, calcium chloracetate, sodium acetate, calcium carbonate, calcium sulphide, and sodium sulphide. These compounds were all added to a 50:50 meat-water bait, at the rate of 3 per cent. of the total weight of the bait. The baits were kept in Mason jars in a laboratory room where the temperature remained in the vicinity of 65 to 70° F. The pH determinations were made regularly on these baits for a period of fourteen days. The results indicated that borax was the most satisfactory buffer, keeping the pH at 8·2-8·3.. Disodium phosphate was nearly as successful, keeping the pH at 7·5-7·7, whereas calcium carbonate was also fairly effective at buffering the bait at 7·8 to 8·0; both of these compounds could, however, not prevent an initial drop in pH in the first two days, corresponding to a similar but sharper drop in the control bait. Calcium chloracetate was very effective in buffering the bait at 5·6; it also acted as a preservative, as also did borax, thus preventing the baits from becoming very attractive.

At a later stage sodium carbonate was also employed (at 0·8 per cent.) in conjunction with other chemicals designed to produce attractive odours (e.g., cystine and phenothiazine). In this series pH measurements were also recorded regularly, and it was shown that sodium carbonate also acts as an effective buffer, at that concentration keeping the pH at 7·9 to 8·0.

This investigation could not be pursued very far, but the general conclusion appears to be that attractiveness does not depend on pH alone. The composition of the chemicals added also have an important bearing on the problem; e.g., where calcium and sodium sulphides were added to meat a pH of 12 was recorded, yet the baits were attractive. It is not clear whether here sulphur-containing gases were solely responsible for the attractiveness.

These experiments, which were only tentative, convinced us that under field conditions reliable data to which statistical methods of analysis could be applied, cannot be obtained unless several replicates are run concurrently and repeated. Certain trap positions are more favourable than others and repeated interchanging of trap positions does not eliminate this factor. By increasing the replicates in each experiment there will be a corresponding increase in the practical difficulties in handling the traps and fly catches. With a limited number of traps, therefore, the logical procedure is to reduce the number of test substances in any one experiment to two: with four replicates of each bait, including the control, twelve traps, therefore, will

be required. The handling of such a number of traps, and of the fly catches entails a considerable amount of work and would virtually be a full-time proposition. As other lines of work offered more promising possibilities it was decided not to proceed with these experiments for the time being.

It was planned, therefore, to carry out olfactory tests with chemically treated carrion in the laboratory and to return later, if necessary, to field experiments.

Toxicity experiments to test the effect of poisoned carcasses on flies and blowfly larvae were planned to be done under laboratory conditions.

Conclusions.

1. The pH of opened carcasses could not be controlled satisfactorily by dusting chemicals on them; but it is also very unlikely that better results will be obtained by the use of solutions of chemicals instead of powders.

2. In view of the above finding is was not found possible to maintain the pH of carcasses at a stage attractive to flies.

3. The attractiveness of carcasses and meat baits appears to depend on a number of factors, and also on the nature of any added chemical. The pH-level alone does not appear a sufficient criterion for attractiveness.

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We wish to acknowledge the co-operation of Dr. H. O. Mönnig and his helpful suggestions in all the various phases of the investigation reported here and in other papers of this series. Furthermore, we tender our thanks to Drs. E. M. Robinson and Max Sterne for preparing bacterial cultures and to Dr. H. L. de Waal for his chemical work on *Stapelia flavirostris* flowers.

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Sheep Blowfly Research V.—Carcasses as Sources of Blowflies.*

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In April 1940 an investigation of certain phases of the sheep blowfly problem was commenced at Onderstepoort by the writer. One of these phases concerned the *rôle* carcasses played in nature as sources of blowflies.

Blowflies possess great powers of reproduction, and an important means of control should be directed against their breeding places. These flies are capable of breeding in carcasses to an enormous extent and also on the living sheep. An effective means of destroying blowfly larvae on the living sheep has been described in article No. VI of this series. The destruction of carcasses has been advised by the Union Department of Agriculture and Forestry for several years.

During the past ten or twelve years much work has been done, especially by Australian investigators, on the ecology of blowflies, and valuable and interesting facts concerning the larval habits of these insects have been obtained. Of the facts brought to light those concerning the phenomenon of succession and competition of the inhabitants of carrion are of fundamental importance. Their experiments in many instances yielded surprising results which revolutionised the ideas held on the relationship of carcasses to the blowfly problem.

The general conclusions of these researches were that the chief factor limiting the numbers of blowflies emerging from a carcass was the competition between the larvae for available food, and that, in summer, larvae of the primary blowfly *Lucilia cuprina* Wied., which began feeding on the carcass during the first day or so after the animal's death, could complete their development. Later arrivals found the carcass overcrowded with secondary and other larvae. They suggested that, for carcass destruction to be successful in reducing the primary blowfly population, it should be done within the first three days after the death of the animal.

In Australia there are at least ten species of flies which are connected with the blowfly problem and which play important *rôles* in the carrion complex.

Although we have fewer species concerned with the problem in South Africa, it was thought that valuable results would be obtained by studying it in the light of Australian experience.

In the Union of South Africa there are several species of blowflies which attack sheep. These are *Lucilia cuprina* Wied., *Lucilia sericata* Meig., *Chrysomyia chloropyga* Wied. and *Chrysomyia albiceps* Wied. A description of these flies is not necessary in an article of this nature, and those interested will find full descriptions in entomological literature.

* See footnote to article I of this series.

Largely as a result of work done in Australia, where the sheep blowfly problem is perhaps a more complex one than in this country, certain blowflies were regarded as primary, others secondary, and some even tertiary. This situation has now come to be recognised in this country. A primary blowfly is one which initiates an attack on a sheep and a secondary is one which follows in the wake of the primary, setting up further infestation.

In the Union of South Africa *Lucilia cuprina* is the more important primary fly and *Chrysomyia chloropyga* the second. *Chrysomyia albiceps* is a secondary fly which, under certain conditions, causes grave injury to sheep. *Lucilia sericata* has been found to cause myiasis but not very commonly. When it is found infesting sheep it is more often in partnership with *L. cuprina* or *Ch. chloropyga*. There have been records of the four species of larvae taken from individual sheep but these are usually the result of neglect of the animals.

The distribution of blowflies and their seasonal incidence in South Africa has been dealt with by other workers, notably by Smit, in various publications. It is only comparatively recently that the two species of *Lucilia cuprina* and *L. sericata* have been separated, so that, in previous publications on blowflies in South Africa, most of the statements confined to *sericata* should be taken to refer to *cuprina*.

During the past three years data on the flies causing strikes have been accumulated. Records were obtained in detail from Onderstepoort and the experiment station at Dohne, C.P. and specimens have been sent by many farmers from all the provinces of the Union. The results showed that *L. cuprina* was responsible for 55 per cent. of the strikes, and 35 per cent. of the strikes were caused by *L. cuprina* in association with the other species. In only 10 per cent. of the cases was *L. cuprina* not implicated (*vide* article I of this series).

Any measures, therefore, designed to control *L. cuprina* would be of considerable importance. Inasmuch as these flies are known to be feeders and breeders in carrion it is reasonable to regard such as important sources of blowflies.

In the past various methods for the disposal or destruction of carrion have been suggested and, in some instances, " carcass " traps designed to lure the flies and to destroy the developing larvae have been utilised.

Besides those species of flies already mentioned certain others, *viz. Chrysomyia marginalis* Wied., *Sarchophaga haemorrhoidalis* Meig., *Calliphora croceipalpis*, various *Musca spp.* and some *Anthomyiaed* flies are attracted to carrion. Of these *Ch. marginalis*, or the large blue bottle fly, is at certain times of the year, particularly in summer, attracted in great numbers to carcasses. This fly is not a sheep blowfly but rare instances of it having caused myiasis in other animals have been known. Its value as a scavenger was shown to be appreciable in the experiments about to be described.

The main object of the experiments was to find out what species of flies bred in carcasses under field conditions.

Methods.

The apparatus: In the first experiment a forty-four gallon oil drum was cut in half lengthwise and each half filled with sand to a depth of about six inches. Two sheep were killed and placed on the sand, one in each drum.

These were exposed for three and ten days respectively and then covered with a wire gauze fly screen to exclude further visitors. After a few days the soil was sifted, larvae and puparia removed to the insectary, and the remaining larvae and puparia adhering to the carcasses, were left *in situ* to develop. The resulting flies were trapped as they emerged from the troughs.

This apparatus was not very suitable because the maggots could escape easily; furthermore, as the troughs were on the ground ants, *Pheidole megacephala*, were able to get in and remove the larvae and also destroy the flies in the traps placed on top of the screen covers.

After experiencing these difficulties with the oil drums larger rectangular metal troughs were made with the edges turned inwards and downwards at an angle of 45 degrees and having a " fence " of wire gauze soldered on and lying parallel to the sides of the trough. This was found to prevent to a high degree the escape of the maggots. To obviate the difficulty with the predatory ants these troughs were placed on platforms raised about twelve inches above the ground and the supports were painted with tanglefoot. The corners of the troughs each had a metal tube attached opening into the troughs at the level of the sand on which the carcasses rested. The wandering larvae could escape through these openings and fall down the tubes into tins containing sand. This sand was usually sieved twice a day and the collected larvae removed to insect boxes constructed to exclude parasites.

Owing to wartime economy the platforms and supports were made of old rough timber which, in the extreme heat of summer, were very difficult to be kept effectively treated with tanglefoot as this quickly melted and ran off leaving conditions suitable for the ants to cross. Almost daily applications of tanglefoot were required to prevent access to the troughs by the ants. These difficulties were finally overcome by standing the platforms in concrete pots containing water. For other reasons too, which will be mentioned later, new and larger troughs of the same type were used. These metal troughs are 54 in. long, 36 in. wide and 21 in. deep and the exit holes 1 in. in diameter in each corner. The depth of sand is 8 in. in each trough.

The former mesh screen covers were re-designed. The latest cover consists of a wood and metal lid which fits closely over the top of the trough. A portion 30 in. by 18 in. in the centre is cut out and over this is built a framework 9 in. high whose sides are covered with wire gauze and the top with metal. This top has a circular hole cut out through which emerging flies can escape into a trap placed over it. To induce the flies to leave the trough strips of cardboard are tacked on the gauze of this top screen. The purpose of this top screen is to provide ventilation which is essential when the carcass is overcrowded with maggots. A photograph (Fig. 1) of the apparatus appears on the next page.

The running of an experiment: Two sheep are used in each experiment, one being exposed for a longer period than the other. This was done to ascertain the differences in the final fly populations in the one instance where competition was unchecked and in the other where later ovipositing fly visitors were excluded from the carcass. During the cooler months carcasses were usually exposed for three and ten days respectively, but in summer when decomposition and the growth of the larvae is extremely rapid, exposures of one and three days, sometimes less, were made.

At frequent intervals after the carcasses were exposed observations on the flies visiting and ovipositing were recorded. The larvae which wandered from the carcasses and escaped through the corner tubes were collected twice a day from the sand tins. In winter, some days after the troughs have been closed the sand is sieved and the larvae and pupae removed to a warm room in the laboratory. The remains of the carcasses containing adhering larvae and pupae are left in the troughs and the flies emerging later are trapped. It would have been an advantage to have suitable containers for the carcass remains to be placed in a warm room but these unfortunately were not obtainable.

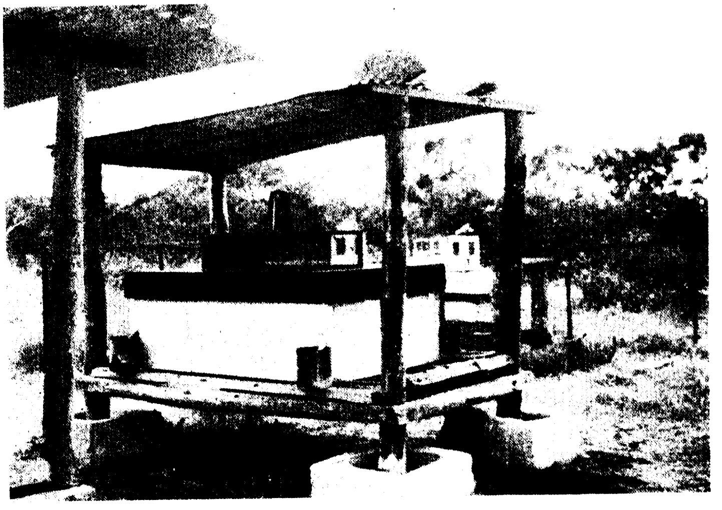

Fig. 1.—*Carcass trough*.—The trough is shown with the lid on. Note the wire gauze screen on the lid and the fly trap above. Migrating larvae escape through the corner tubes and are collected in the tins containing sand. During the exposure of the carcass the lid and corrugated iron roof are removed.

Parasites and predators, except ants, had access to the carcasses. Those larvae which escaped from the troughs into the sand tins were regarded as being successful in avoiding parasites and predators, and were subsequently protected under laboratory conditions.

Under natural conditions larvae migrate considerable distances from a carcass, thereby reducing the chances of parasitism, especially by *Mormoniella vitripennis* Wlk., which oviposits particularly in exposed puparia. In an experiment of this type, therefore, everything should be designed to simulate as closely as possible natural conditions, hence the tubes permitting the escape of wandering maggots. Further, the reason for using large troughs was to have sufficient space between the carcass and the walls of the trough. In smaller troughs the animal just fitted in touching all sides. When a carcass is swarming with maggots this cramping tends to disperse the maggots unduly, thereby introducing an undesirable factor which the larger troughs obviated. The sand on which the carcasses are placed is 9 in. deep, thus allowing a volume great enough to accommodate the pupating larvae. Should the sand be too shallow a great proportion of the larvae would be forced to pupate on the surface thereby being unduly exposed to parasitism.

In some instances the sheep were killed by cutting their throats, but in the majority of the experiments, the animals were shot in the head by means of a humane killer and the wounds plugged immediately with wads of wool taken from the animals. It was felt that the cutting of the throats and the subsequent haemorrhage might have some unusual effect on the process of decomposition and the resulting odours, which may not be normally associated with carcasses of animals dying from natural causes. However, under field conditions there would be deaths from jackal attacks and partly eaten carcasses would be left on the ground. Although death by shooting in the brain would not simulate natural death, bleeding is reduced to a minimum. It has been observed that fresh blood attracts blowflies and that flies are attracted more quickly to a carcass which has been opened to expose the viscera .

Apart from the experiments with sheep carcasses a number of small animals, e.g., rats, snakes, fowls, rabbits, guinea-pigs and cats were killed and their bodies exposed to flies. These small carcasses were placed in suitable containers with sand, and after they were broken down by larvae they were placed in insect boxes and removed to the laboratory. The results obtained from these are shown in Table 3.

Results.

(*a*) *Sheep carcasses:* The results of the experiments at Onderstepoort with whole carcasses are shown in Table 1.

A parallel set of experiments carried out by A. H. de Vries, Entomologist at the Grootfontein College of Agriculture, Cape Province, has been in progress for over a year. Some of these results (to be published more fully later) are shown in Table 2.

In the Onderstepoort experiments the wandering larvae were unable to escape in experiments two, three, and four, as at that time the exit tubes had not been attached to the troughs. In the first experiment where a half oil drum was used many larvae escaped but the numbers are not shown in the table. In the other experiments where migration has been recorded it will be noted what dimensions it assumes.

From Table 1 the most striking results may be stated: *Lucilia* species breed in carcasses during the winter and cooler months but not in summer.

Ch. chloropyga does not breed from carcasses in summer.

Ch. marginalis and *Ch. albiceps* are particularly successful in summer while the former is not bred to any great extent in winter.

In several instances the longer the exposure in summer the greater the percentage of *Ch. albiceps* in the final emerging population.

From Table 2 the results show: An enormous population of *Lucilia* spp. and *Ch. chloropyga* during winter and a decrease of these species as the weather becomes warmer. In midsummer *Ch. marginalis* and *Ch. albiceps* constitute the population obtained from the carcasses. Unlike some of the results obtained at Onderstepoort, the proportion of *Ch. marginalis* is greater than that of *Ch. albiceps* the longer the carcass is exposed.

It would appear from a comparison of the results from the two tables that *L. cuprina*, *L. sericata* and *Ch. chloropyga* may be bred from carcasses for a greater portion of the year at Middelburg in the Karroo than at Onderstepoort which is sub-tropical in climate. In this respect it is interesting to compare these results with those obtained in Australia. In that

country it was noted that *Chrysomyia* spp. flies bred more successfully in the warmer climate of Queensland than in the cooler climate of New South Wales, and that in the far northern territory *Chrysomyia* spp. and *Sarcophagids* constituted the dominant species of the population emerging from carrion.

(*b*) *Small animal carcasses:* Unfortunately there were not enough exposures of these carcasses in the summer but this was unavoidable due to the writer's absence from the institution for three months at that time. The few summer exposures show an absence of *Lucilia* spp. and *Ch. chloropyga*, while, during the winter, these species bred successfully in small carcasses. *Ch. marginalis* and *Ch. albiceps* constitute the dominant species in the summer populations from these carcasses.

From the available records, therefore, it seems reasonable to suppose that fly populations from small and big carcasses are very similar in composition.

It is interesting to note what enormous numbers of flies relative to the available food supply are produced. From a small *Otomys* rat barely four inches long 730 *L. sericata* were obtained, and from a small cat containing 640 gm. of available food 7,933 blowflies were bred.

In Australia Fuller noted that different kinds of animal carcasses yielded different species of larvae, e.g., "a cat always has many more *Chrysomyia* spp. larvae present than a guinea-pig, whilst the guinea-pig may contain only a few dozen hairy (*Chrysomyia*) maggots and be crowded with those of *Lucilia* spp. . . . ." She also notes that pieces of beef seldom contain "hairy" maggots.

Our records are not complete enough to draw conclusions on this aspect. In order to obtain significant results it would be necessary to expose simultaneously about six carcasses of each kind of animal and to repeat the experiment at different times of the year.

The available records of populations from small carcasses show great variation in the same kinds of animals. The explanation is not obvious and it is suggested that it is largely a matter of chance. For example, two cats of the same variety were exposed about thirty feet apart; from the one over 1000 *L. sericata*, 6000 *Ch. chloropyga* and no *Ch. marginalis* were bred, and from the other no *L. sericata*, very few *Ch. chloropyga*, but nearly 2000 *Ch. albiceps* and 100 *Ch. marginalis*. The latter carcass began to attract flies to oviposit about five days after the former.

A salient point to be noted from these records is that small carcasses can produce many blowflies and, most important, many primary flies, e.g., 344 *L. cuprina* from a small *Otomys* rat.

In all these experiments the phenomenon of succession already referred to was noted. In summer the first flies to arrive, sometimes in less than an hour after the death of the animal, were *L. cuprina* and *L. sericata* followed almost immediately by *Ch. marginalis*. The next to come is *Ch. chloropyga* and then *Ch. albiceps*. *Musca* spp. usually arrive when the carcass has been broken down and are presumably mainly attracted by the exposed contents of the digestive organs. Sarcophagid species also arrive in the later stages of decomposition. There is an overlapping in the succession and in winter succession does not operate. The fact that *Ch. marginalis* is a very early visitor to carcasses is important for this fly lays prodigious masses of eggs at about the same time as the Lucilias.

Competition amongst the maggots is extremely keen though from these experiments a complete picture of the different stages cannot be obtained. Larvae of *Ch. marginalis* are large and very active, and in summer they swarm over the carcasses in great numbers. They possibly prey on other larvae and probably account for the destruction of the larvae of Lucilias and *Ch. chloropyga* during summer. Holdaway (1930), working in France on the insects inhabiting carrion, states that the greatest reduction in summer in the numbers of *L. sericata* is brought about by *Ch. albiceps*. He gives an example, a rabbit (1 Kg. of available meat) exposed during summer and having an initial population of 60,350 *L. sericata* and 2,850 *L. caesar* yielded a final population of 30 Lucilia and 2,611 Chrysomyia.

In Australia, Fuller records Chrysomyia larvae driving off and overwhelming larvae of *Lucilia* spp. and *Calliphora* spp. The larvae of *Sarcophagidae* and *Musca* spp. play a very minor *rôle* in competition and for all practical considerations may be ignored. Various beetles, mostly *Dermestes vulpinus* and several species of *Histeridae* are attracted to carcasses, feeding to a small degree on larvae and puparia but not appreciably affecting the final results. Parasitism by *Mormoniella vitripennis*, Wlk., occurs more particularly on *Ch. albiceps* and *Ch. chloropyga*. Lucilia larvae which are the first to migrate are more successful in escaping from this parasite. Where Lucilias have been obtained from sheep carcasses the great majority have been reared from larvae which have migrated. This migration occurs in the early stages of the experiments. In summer, when no Lucilias are obtained, it seems reasonable to suppose that larvae of *Ch. marginalis*, which are present soon after the death of the animal, devour the former or else render the pabulum otherwise unsuitable to them.*

Practical considerations from the above indicate that measures should be adopted to dispose of carcasses particularly when they breed Lucilias and *Ch. chloropyga*. During the period of the year when *Ch. marginalis* is most abundant and Lucilias are not successful in carrion the destruction of carcasses is not so important, except in cases of infectious disease. On the other hand *Ch. albiceps*, a secondary sheep blowfly, is a highly successful breeder in summer carcasses particularly in those left exposed for three days or longer. It would be desirable, therefore, to design methods to foster *Ch. marginalis*. This might be accomplished in summer by burying carcasses three days after the death of the animal. During this time any Lucilia and *Ch. chloroypga* larvae present would be eliminated by the active *Ch. marginalis*, while, in most instances, the later arriving *Ch. albiceps* would, therefore, be excluded.

It is interesting to note the effect of burying carcasses containing maggots. Smit has shown how successful are *Ch. chloropyga* and Lucilia maggots in burrowing upwards through six feet of soil. Fuller has shown that burial has a deleterious effect on larvae of *Ch. ruficacies* and this would probably apply to *Ch. albiceps*, a closely related fly and almost identical in appearance. The effect of burial on larvae of *Ch. marginalis* has not been determined. The simple burial of a carcass favours the primary species. This was strikingly demonstrated by Fuller. Burial must be combined

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* Since this work was done tentative experiments conducted by Dr. Ulyett at the Entomological Parasite Laboratory, Pretoria, have failed to demonstrate any preying on larvae of *Lucilia* spp. by those of *Chrysomyia marginalis*. Larvae of *Ch. albiceps*, on the other hand, were shown to prey very actively on *Lucilia* larvae. Sufficient data to enable conclusions to be drawn have not yet been obtained.

with the poisoning of carcasses to control the emergence of primary flies at such times of the year when these flies are usually successful breeders in carrion.

Experiments on similar lines to these have been commenced in a coastal district of the Cape Western Province. The climate is different from that of the rest of the Union and a comparison of blowfly populations from carcasses there and from other parts of the country will be interesting.

CONCLUSIONS.

1. The object of the investigation was to determine what species of flies bred in carcasses exposed under field conditions.

2. The technique of exposing sheep carcasses in specially constructed troughs and the collection of migrating larvae and the trapping of emerging flies are described.

3. Fly populations from small animal carcasses were bred at various times of the year.

4. The phenomenon of succession and competition of the inhabitants of carrion was demonstrated.

5. *Lucilia cuprina*, *Lucilia sericata* and *Chrysomyia chloropyga* mainly constituted the populations of flies bred during the cool time of the year, while *Chrysomyia marginalis* and *Chrysomyia albiceps* constituted the populations during mid-summer.

6. When *Ch. marginalis* is abundant it is attracted to carcasses in the first stage of decomposition. *L. cuprina* and *L. sericata* are usually the first visitors to fresh carcasses followed almost immediately by *Ch. marginalis*.

7. Certain suggestions are made for the treatment of carcasses as a means of controlling blowflies.

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TABLE 1. *Blowflies Bred in Sheep Carcasses at Onderstepoort.*

Experiment Number.	Date when Experiment Commenced.	No. of Days Carcass Exposed.	Flies Obtained.							Remarks.
			Lucilia cuprina.	*Lucilia sericata.*	*Chrysomyia chloropyga.*	*Chrysomyia albiceps.*	*Chrysomyia marginalis.*	Other Species.	Total Number of Flies.	
1	5/ 6/40	3	460	238	22,358	1,359	43	—	24,458	Carcasses exposed in half oil drums.
		10	13	43	17,253	14,054	2,044	—	33,407	Large number of *Lucilia spp.* probably lost.
2	15/11/40	3	—	—	—	5	19,836	541	20,382	On the third day the carcasses were almost totally destroyed.
		7	—	—	—	16,477	6,435	226	23,138	
3	3/ 2/41	1 day 5 hrs.	3	4	—	1,337	16,039	283	17,666	Abdomens opened to expose contents.
		2 days	—	—	—	7,960	14,249	699	22,908	
4	2/ 6/41	3	475	—	24,951	2,861	4,203	720	33,210	
		7	—	—	4,635	4,350	36	69	9,090	
5	20/ 8/41	3	174	447	9,016	77	150	—	9,864	Flies from larvae which had migrated.
			—	—	6,467	659	62	53	7,241	Flies emerged in carcass trough.
		TOTAL.....	174	447	15,483	736	212	53	17,105	
		13	405	834	12,813	4	361	—	14,417	Migrated.
			6	4	2,652	978	41	194	3,875	From trough.
		TOTAL.....	411	838	15,465	982	402	194	18,292	
6	30/ 9/41	2	—	—	—	13,062	6,237	—	19,299	Migrated.
			—	—	—	6,273	2,707	6	8,986	From trough.
		TOTAL.....	—	—	—	19,335	8,944	6	28,285	
		7	—	—	—	9,230	30,727	—	39,957	Migrated.
			—	—	—	166	505	12	683	From trough.
		TOTAL.....	—	—	—	9,396	31,232	12	40,640	

TABLE 1.—(*continued*).

Experiment Number.	Date when Experiment Commenced.	No. of Days Carcass Exposed.	Flies Obtained. *Lucilia cuprina.*	*Lucilia sericata.*	*Chrysomyia chloropyga.*	*Chrysomyia albiceps.*	*Chrysomyia marginalis.*	Other Species.	Total Number of Flies.	Remarks.
7	31/10/41	1	—	—	—	10,245	424	—	10,669	Migrated.
			—	—	—	3,989	166	—	4,155	From trough.
		TOTAL.....	—	—	—	14,234	590	—	14,824	
		7	—	—	—	6,485	4,050	—	10,535	Migrated.
			—	—	—	2,506	870	—	3,376	From trough.
		TOTAL.....	—	—	—	8,991	4,920	—	13,911	
8	3/12/41	1	—	—	—	6,294	9,395	—	15,689	Lamb's carcass.*
			—	—	203	1,918	7,821	—	9,942	Lamb's carcass. Eggs and larvae removed and bred on meat kept in insect boxes.
		3	—	—	—	1,427	1,488	—	2,915	Lamb's carcass.
9	17/ 3/42	1	33	—	—	—	12,445	—	12,478	Migrated.
			24	5	98	7,284	6,964	112	14,487	From trough.
		TOTAL.....	57	5	98	7,284	19,409	112	26,965	
		5	—	—	1	2,724	10,209	—	12,934	Migrated.
			—	1	3	11,912	3,560	101	15,577	From trough.
		TOTAL.....	—	1	4	14,636	13,769	101	28,511	

* In this experiment a third carcass was included in order to obtain eggs and newly emerging larvae for rearing in an environment when larval competition was greatly reduced. In this way it was hoped to determine what species of larvae were present in the first twenty-four hours of exposure without resorting to the method of examining first instars individually. Furthermore, there is no satisfactory way of distinguishing between the early instars of *L. cuprina* and *L. sericata*. It is interesting to note that *Ch. chloropyga* were recovered only from the eggs and larvae which were removed before intense competition set in. Carcasses of lambs were used to reduce the labour entailed in working with large carcasses.

TABLE 1.—(*continued*).

Experiment Number.	Date when Experiment Commenced.	No. of Days Carcass Exposed.	Flies Obtained. *Lucilia cuprina.*	*Lucilia sericata.*	*Chrysomyia chloropyga.*	*Chrysomyia albiceps.*	*Chrysomyia marginalis.*	Other Species.	Total Number of Flies.	Remarks.	
10	23/ 4/42	2	196	39	3,035	49	432	1	3,752	Migrated.	*Ch. marginalis* and *Ch. albiceps* visited carcasses in first day; on second day *Ch. marginalis*, *Ch. albiceps*, *L. cuprina*, *L. sericata* and *Ch. chloropyga*.
			28	29	1,175	8,769	7,885	119	18,005	From trough.	
		TOTAL.....	224	68	4,210	8,818	8,317	120	21,757		
		6	6	1	—	63	972	—	1,042	Migrated.	
			—	—	—	15,587	2,980	1	18,568	From trough.	
		TOTAL.....	6	1	—	15,650	3,952	1	19,610		
11	23/ 6/42	4	1,516	1,025	—	—	—	—	2,541	Migrated.	Very cold weather during first five days. Blowflies began to arrive on the second day.
			272	1,313	500	1	—	18	2,104	From trough.	
		TOTAL.....	1,788	2,338	500	1	—	18	4,645		
		14	124	10,293	473	—	—	125	11,015	Migrated.	
			125	2,235	22,505	62	2	93	25,022	From trough.	
		TOTAL.....	249	12,528	22,978	62	2	218	36,037		

TABLE 1.—(*continued*).

Experiment Number.	Date when Experiment Commenced.	No. of Days Carcass Exposed.	Flies Obtained.							Remarks.
			Lucilia cuprina.	*Lucilia sericata.*	*Chrysomyia chloropyga.*	*Chrysomyia albiceps.*	*Chrysomyia marginalis.*	Other Species.	Total Number of Flies.	
12	5/ 8/42	3	87	310	13,755	95	2,233	—	16,480	Migrated.
			12	30	32,380	3,770	9,295	—	45,487	From trough.
		TOTAL.....	99	340	46,135	3,865	11,528	—	61,967	
		12	376	2,825	12,094	4	147	—	15,446	Migrated.
			15	140	28,150*	2,934*	2,247*	40	33,526	From trough.
		TOTAL.....	391	3,965	40,244	2,938	2,394	40	48,972	
13	25/ 9/42	3	—	—	1,580	11,541	6,810	6	19,937	Migrated.
			—	—	10	1,752	1,476	55	3,293	From trough.
		TOTAL.....	—	—	1,590	13,293	8,286	61	23,230	
		7	—	—	2	1,133	15,420	1	16,556	Migrated.
			—	—	—	7,130	1,270	—	8,400	From trough.
		TOTAL.....	—	—	2	8,263	16,690	1	24,956	

* Many of these species had escaped from trough.

TABLE 2. *Blowflies Bred in Sheep Carcasses at Grootfontein College of Agriculture: Middelburg, C.P.*

Experiment Number.	Date when Experiment Commenced.	No. of Days Carcass Exposed.	Flies Obtained: *Lucilia cuprina.*	*Lucilia sericata.*	*Chrysomyia chloropyga.*	*Chrysomyia albiceps.*	*Chrysomyia marginalis.*	*Calliphora croceipalpis.*	*Sarcophaga* spp.	Other Species.	Total Number of Flies.	Remarks.
1	4/ 7/41	3	6,502	8,128	759	—	—	107	22	—	15,518	
		10	15,371	25,948	23,925	28	—	162	24	—	65,458	
2	6/ 8/41	3	17,183	17,004	510	—	—	—	7	64	34,768	
		10	25,767	20,589	47,228	—	—	30	—	—	93,614	
3	11/ 9/41	3	12,180	9,214	37,724	—	—	—	11	—	59,139	
		10	896	633	38,226	97	—	—	—	—	39,852	
4	10/10/41	3	863	1,676	52,916	—	—	—	22	—	55,477	
		10	977	899	21,234	438	—	—	—	—	23,548	
5	10/11/41	3	—	8	8,954	2,130	—	—	—	573	11,665	
		10	14	—	17,343	7,213	—	—	4	—	24,574	
6	13/12/41	3	—	—	—	16,140	12,839	—	—	—	28,979	
		10	—	—	—	12,080	27,397	—	—	—	39,477	
7	14/ 1/42	3	—	—	4	16,084	6,232	—	—	—	22,320	
		10	—	—	—	11,811	37,829	—	—	—	49,640	
8	12/ 2/42	3	—	—	11	10,521	20,153	—	—	—	30,685	
		10	—	—	50	7,923	25,650	—	—	1,698	35,321	
9	14/ 3/42	3	—	—	—	7,949	24,090	—	—	—	32,039	
		10	—	—	9	2,419	4,544	—	—	63	7,035	Parasitism was marked.
10	9/ 4/42	3	—	—	40	620	592	—	—	11	1,263	Many larvae escaped from insect boxes.
		10	—	—	—	5,656	11,226	—	—	16	16,898	
11	15/ 5/42	3	10,142	2,659	5,995	766	361	23	23	225	20,194	
		10	2,886	1,368	15,133	4,038	2,119	—	—	17	25,561	
12	18/ 6/42	3	24,795	5,182	7,408	38	—	77	115	690	38,305	
		10	23,283	6,224	15,089	89	—	45	45	1,522	46,297	

TABLE 3.

Flies Bred from Small Carcasses.

Carcass.	Period Exposed.	Flies Bred.	
Rabbit	17/11/41–22/11/41	*Ch. albiceps*	2,971
		Ch. marginalis	448
Mouse	29/10/41– 2/11/41	*Sarcophaga haemorrhoidalis*	33
Guinea-pig	29/10/41– 5/11/41	*Ch. albiceps*	300
Guinea-pig	21/ 8/41– 2/ 9/41	*L. cuprina*	13
		L. sericata	35
Lamb (at birth)	23/ 8/41–10/ 9/41	*L. cuprina*	14
		L. sericata	1,013
		Ch. chloropyga	1,693
		Ch. albiceps	2,172
		Ch. marginalis	12
Otomys rat	29/ 3/41– 1/ 4/41	*L. sericata*	35
Otomys rat	2/ 4/41– 8/ 4/41	*L. cuprina*	3
		L. sericata	185
		Sarcophaga spp	124
		Musca spp	5
Otomys rat	4/ 4/41–15/ 4/41	*L. cuprina*	6
		Sarcophaga spp	7
Otomys rat	3/ 4/41–10/ 4/41	*L. cuprina*	344
		L. sericata	187
Rat	10/ 4/41–18/ 4/41	*L. sericata*	2,353
		Ch. albiceps	13
Rat	10/ 4/41–15/ 4/41	*L. cuprina*	4
		L. sericata	247
		Ch. albiceps	8
		Sarcophaga spp	60
Rat	10/ 4/41–16/ 4/41	*L. sericata*	383
Rat	10/ 4/41–21/ 4/41	*L. sericata*	378
Rat	11/ 4/41–17/ 4/41	*L. sericata*	50
		Ch. albiceps	341
Rat	10/ 4/41–19/ 4/41	*L. cuprina*	50
		L. sericata	571
Snake	18/ 4/41– 2/ 5/41	*Ch. chloropyga*	341
		Ch. albiceps	1,458
		Ch. marginalis	161
Fowl	19/ 4/41– 2/ 5/41	*L. cuprina*	188
		L. sericata	26
		Ch. chloropyga	11
		Ch. marginalis	2,986
Cat	11/ 5/42–25/ 5/42	*Ch. chloropyga*	28
		Ch. albiceps	1,906
		Ch. marginalis	104
Cat	11/ 5/42–30/ 5/42	*L. sericata*	1,367
		Ch. chloropyga	6,410
		Ch. albiceps	156

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Sheep Blowfly Research VI.—The Treatment of Myiasis.*

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I. General Considerations.

The properties of an ideal dressing for the treatment of blowfly strikes have been enumerated by Lennox (1941) as follows:—

(i) It should be stable.
(ii) It should possess sufficiently low viscosity and surface tension to enable it to penetrate readily into a strike wound and into the surrounding fleece.
(iii) It should not damage the fleece and it should be easily removed in the industrial process of scouring.
(iv) It should resist atmospheric decomposition after application to the sheep and should not be readily removed by rain or urine.
(v) It should kill the maggots in a strike or so injure them that they can do no further damage.
(vi) It should be non-toxic to the sheep.
(vii) It should promote rapid healing of the strike wound.
(viii) It should prevent restrike both during the healing process and subsequently.
(ix) If an aqueous system, the dressing should be easily prepared by adding water to the solid components.
(x) It should be cheap.

These requirements are admirably stated and may serve as a basis for the search after an ideal dressing. However, point (v) requires modification, especially in view of the observations referred to in Article No. V of this series, from which the conclusion must be made that the breeding-place of *Lucilia cuprina* during the summer is on live sheep, at least in South Africa, although this would seem to be also largely the case in Australia. If the phrase "that they can do no further damage" is understood in the absolute sense, i.e. that they will also not become flies which may again strike sheep, it is in order. But if it refers only to damage in the maggot stage on the particular struck sheep, it is insufficient.

In the past most investigators laid more stress on the prevention of re-strike than on the killing of the maggots and all dressings with which the writer is acquainted suffer from this deficiency. The B. T. B. mixture described by Lennox falls in the same category. It is stated that "many of the maggots left the wound as soon as the dressing was applied and the majority of those which remained were killed within 10 minutes". The writer tested all blowfly dressings used in South Africa as well as a few

* See footnote to article I of this series.

others and found in practically all cases that the majority of the maggots left the wound very soon after the application, that these maggots would drop off the sheep and eventually develop into blowflies. In a few cases the maggots were stunned by the dressing and therefore readily dropped off the sheep as soon as the latter was released—when it usually shakes itself or stamps its hindlegs—and the majority recovered in 30-60 minutes, pupated and became flies.

It is obvious that if the fly can breed only on the sheep during several months of the year, the killing of all maggots in strike wounds should markedly decrease the incidence of blowflies or even exterminate them. It is therefore imperative that a dressing should kill 100 per cent. or very nearly that proportion of the maggots. For this purpose a stomach larvicide will not be satisfactory on account of the fact that the majority of the maggots are driven away and are not affected by such a poison, at least not by those so far used for this purpose. A contact larvicide from which the maggots cannot escape must consequently be used, and it should act rapidly before the maggots can get away from it or, if they are stunned, before they may drop off the sheep.

Point (x) of the properties, viz., that the dressing must be cheap, is very important in order to enable every farmer to use it, so that the killing of maggots could be generally and regularly carried out. In the investigations recorded in this paper the question of costs was, therefore, continually kept in mind.

Point (viii), that the dressing should prevent restrike both during the healing process and subsequently, is a high ideal which may be obtained by the inclusion in the dressing of stomach poisons, which would remain effective some time after healing has occurred. But this takes the dressing into the category of prophylactics and one might be satisfied with a dressing which prevents restrike until healing is completed, especially if extended protection should appreciably increase the cost of the dressing and so limit its use.

Protection against restrike can be effected in three ways: (1) by repellents, (2) by larvicides, especially stomach poisons such as boric acid and diphenylamine, or (3) by making the wound unattractive and unsuitable in other ways, based on a knowledge of the factors which tend to favour restrike. Repellents are limited in their effects and lately there seems to be a general tendency amongst investigators on this subject to look for other means of protection. In 1936 the writer reported on a dressing in which carbon tetrachloride was used as larvicide in an aqueous emulsion with wool-grease, to which Tagetes oil was added as a repellent. Tagetes oil was more effective than pine tar oil which, until then, had been considered the best blowfly repellent. But later—Mönnig (1940)—it was stated that even this oil was not sufficiently effective during a bad strike wave and " that it would be far better to get the wounds to dry and heal rapidly and also to make them unattractive and unsuitable for restrike in other ways than by means of repellents ". The above-mentioned emulsion also suffered from another defect which is common to all aqueous dressings but which was only realised at a later stage. This matter will be discussed presently.

Larvicides as protectives have great possibilities, especially because their use would tend to extend protection beyond the healing stage. Manufacturers of proprietary dressings continue to incorporate arsenical compounds for this purpose, but they are undesirable on account of their

irritating and poisonous properties when used on a wound or a raw surface. In building up the blowfly spray described in this article, the writer seriously considered the incorporation of boric acid or other larvicides of this type and it may still be desirable to add such an ingredient, but under present international conditions the difficulty of obtaining the necessary chemicals and the increase in costs which would result led to the decision to do without them.

In 1940 the writer reported on tests carried out with wattle-bark extract (mainly tannates of catechol and pyrogallol) and discussed the reasons for attractiveness on which the use of this substance was based. Briefly the facts are that alkalinity and moisture are the main essential factors and consequently it was concluded that a wound should become unattractive to blowflies and unsuitable for young maggots if it was acidified and made to dry rapidly. Hence the use of an acid astringent.

In pursuance of this line of investigation further tests were made and several facts emerged on account of which the use of wattle-bark extract was suspended in favour of other substances. As previously reported, an emulsion of benzol in aqueous wattle extract solution attacks metal and, therefore, the two substances were applied separately and successively, which meant a double treatment against which several farmers, who carried out tests, raised objections. Moreover it was found that, in a certain percentage of cases, restrikes occurred, not on the site of the original strike but immediately next to it where none of the material had been applied. At first it was thought that this was due to failure to treat the whole strike, but it was soon realised that another factor was responsible and that this might be a defect inherent in all aqueous dressings, especially when it was recalled that similar restrikes had been reported after the use of the carbon tetrachloride-Tagetes oil emulsion referred to above. It now appears very probable that an aqueous dressing may provide a source of moisture, from which the suint in the immediate vicinity draws water and that in this way new attractive foci may be set up leading to strikes, just as happens in the case of soiling by diarrhoeic faeces. For these reasons the aqueous wattle extract was dropped, although further but unsuccessful attempts were later made to incorporate alcoholic solutions into other mixtures. It was decided to obtain the acid, astringent and drying effects by other means and alcohol was thought of particularly as a possible astringent and drying agent.

II. Tests for Larvicidal Efficacy.

A further fact which emerged from the work on wattle extract dressings was that *in vitro* tests of dressings for larvicidal value, at least where contact larvicides are concerned, are misleading and valueless. In the preliminary report referred to it has been recorded that emulsions of benzol in aqueous wattle extract solutions were 100 per cent. effective in killing maggots *in vitro* in 1 to 3 minutes. The further tests then reported on were mainly concerned with the efficacy of such emulsions in preventing restrikes. When tests of the larvicidal effect were subsequently carried out on actual strikes it was found that the results fell far short of what had been expected on the basis of *in vitro* tests. The average larvicidal efficacy obtained in several tests was only 63 per cent. Similar differences between the results of *in vitro* and *in vivo* tests were later noted with other mixtures and therefore the *in vitro* method was discarded. All tests of larvicidal efficacy recorded in this paper were made on artificial strikes. Three days after application of the first stage larvae, when the maggots were full-grown and almost ready to

leave the sheep, the strike area was clipped, the dressing to be tested was poured or sprayed on and time was kept by means of a stop-watch. From one to three minutes after application of the dressing maggots were removed and placed on clean sand in glass containers. The latter were covered with gauze and placed in an aquarium room with a moderately warm and moist atmosphere. About 14 days later, when all flies that would emerge had died, the content of the vessel was passed through a sieve and the results recorded as dead larvae, dead pupae and flies emerged. The reason for the difference in results obtained by the two methods is not clear; possibly the larvae in a wound are to some extent protected by a covering layer of the substances which surround them and very probably the alkalinity of the medium plays a part, as is indicated by the effect of acid ingredients in dressings to be mentioned later.

III. Selection of Larvicide.

On the basis of these general considerations an attempt was made to build up a dressing. The first requirement was a suitable larvicide. Pyrethrins, nicotine, paradichlorbenzene, and several other insecticides appeared to have no or very little effect on the maggots. Carbon tetrachloride is too expensive. Attention was, therefore, directed to coal-tar distillates of which some were known to be very effective.

Benzol is rather irritating to sheep or, at least, causes pain on application and it was thought that fractions with higher boiling points and larger molecules may be less irritating. Preliminary tests of such fractions showed that the irritating effects of some of them were due mainly to tar acids (phenol and cresols), ta bases (quinoline and isoquinoline) and naphthalene which they contained. Therefore, these fractions were obtained free of acids and bases for further tests. It was also found that the addition of naphthalene did not increase the efficacy of any of these fractions, but that the larvicidal properties were indeed lowered by a naphthalene content of over 5 per cent. and consequently the naphthalene was removed from fractions containing it. The tar fractions used in all the following tests were, therefore, neutral and free of naphthalene. The results obtained with the various fractions alone are given in Table 1.

Table 1.

Fraction.	B.P. (°C.).	L.	P.	F.
Benzol	± 80	70	24	6
Xylol	133–134	11	—	89
Naphtha A	120–160	31	34	35
Naphtha 11A	143–160	12	58	30
Naphtha 11	143–167	15	36	49
Naphtha 4	160–180	35	27	38
Naphtha 4, free of cumarone	±160–180	24	39	37
Heavy naphtha	160–200	20	18	62
Naphtha 3	180–200	8	28	64
Creosote oil 6	200–240	68	10	22
Creosote oil 7	240–300	55	14	31
Naphtha 11A : 50, Creosote oil 6 : 50	—	33	23	44

Explanation.—L. P and F are the percentages of dead larvae, dead pupae and flies emerged as obtained by the technique described above. As a rule three strikes were treated with each substance tested, but frequently a larger number of tests were made and the results here given are the averages of all such tests and usually refer to well over 100 maggots.

If a graph is drawn from these figures it will be seen that there are three apices, one produced by benzol, another by the light naphthas boiling at about 140 to 160° C. and a third by the creosote oils boiling at about 200 to 240° C.

IV. Addition of Cresols.

In previous tests made with the same substances which had not been purified of tar acids and bases, it had been noted that the larvicidal effect was better than now obtained. Since a reasonable quantity of tar acid in a dressing would add the advantage of a disinfectant, tests were now made with the same fractions to which the phenol or cresols were added in definite quantities. The results are given in Table 2:—

Table 2.

Fraction.	B.P. (°C.).	Percentage Tar Acid Content.	L.	P.	F.
Benzol	± 80	2·5 Phenol	89	9	2
Benzol	± 80	5 Phenol	82	5	13
Naphtha A	120–160	2·5 Phenol	77	22	1
Naphtha A	120–160	5 Phenol	91	6	3
Naphtha 4	160–180	2·5 Phenol	66	28	6
Naphtha 4	160–180	5 Phenol	55	25	20
Naphtha 3	180–200	5 Phenol	23	60	17
Naphtha 3	180–200	10 Phenol	85	10	5
Creosote oil 6	200–240	2·5 Phenol	25	15	60
Creosote oil 6	200–240	5 Phenol	53	26	21
Benzol	± 80	2·5 Cresol (crude).	52	20	28
Benzol	± 80	5 Cresol (crude).	90	5	5
Naphtha A	120–160	2·5 Cresol (crude).	54	30	16
Naphtha A	120–160	5 Cresol (crude).	98	1	1
Naphtha 11A	143–160	5 Cresol (crude).	24	56	20
Naphtha 11	143–167	5 Cresol (crude).	44	47	9
Naphtha 10	158–170	5 Cresol (crude).	39	46	15
Naphtha 4	160–180	5 Cresol (crude).	49	42	9
Naphtha 3	180–200	5 Cresol (crude).	39	46	15
Creosote oil 6	200–240	5 Cresol (crude).	36	21	43

It seems strange that 5 per cent. phenol tends to make some of the fractions less effective than 2·5 per cent. phenol while cresol has the opposite effect. The results are definitely in favour of light naphtha while creosote oil does not show up well at all.

V. Addition of Drying Agent.

The addition of other ingredients, viz., the drying and astringent agent and the acid were now considered. As previously stated, alcohol had been thought of as a possible astringent and drying agent and its use had meanwhile been more strongly suggested by rather unexpected effects produced by a proprietary dressing which contained alcohol. Mr. M. C. A. Nolte had

also drawn my attention to the articles by Hurst (1940), Trim (1941) and Wigglesworth (1941) in which mention is made of the penetration of oil-alcohol mixtures through the cuticle of maggots, but the significance of this was not realised at the time and the articles were read later when this realisation came through the results of the tests now to be recorded.

First it was desired to determine whether 96 per cent. Ethyl Alcohol alone or in combination with the distillates had any effect on the maggots and the following tests were made:—

TABLE 3.

96 Per Cent. Alcohol.	Tar Distillate.	L.	P.	F.
—	Naphtha 10 : 100	31	38	31
10	Naphtha 10 : 90	15	56	29
20	Naphtha 10 : 80	40	43	17
30	Naphtha 10 : 70	77	10	13
40	Naphtha 10 : 60	96	—	4
50	Naphtha 10 : 50	98	1	1
60	Naphtha 10 : 40	98	1	1
70	Naphtha 10 : 30	97	1	2
80	Naphtha 10 : 20	85	8	7
90	Naphtha 10 : 10	52	19	29
100	—	0	26	74

Although alcohol has little effect on the maggots, it is obvious that it increases the efficacy of the naphtha and that the most effective mixture lies about the 50:50 mark or even a higher proportion of alcohol.

Tests were then made with combinations of equal parts of 96 per cent. alcohol and various neutral tar fractions. Results are shown in Table 4.

TABLE 4.

Fraction.	B.P. (°C.).	L.	P.	F.
Benzol	± 80	98	1	1
Naphtha A	120–160	96	1	3
Naphtha 11A	143–160	74	5	21
Naphtha 11	143–167	88	3	9
Naphtha 10	158–170	98	1	1
Naphtha 4	160–180	87	4	9
Naphtha 3	180–200	88	7	5
Creosote oil 6	200–240	86	5	9

If these results are compared with those given in Table 1 a marked improvement along the whole series is evident.

Combinations of neutral tar fractions with 96 per cent. alcohol and Cresol were now made. The proportions were in each case respectively 47·5 per cent., 47·5 per cent., and 5 per cent; the results are given in Table 5.

TABLE 5.

Fraction.	B.P. (°C.).	L.	P.	F.
Benzol	± 80	94	5	1
Naphtha A	120–160	85	3	12
Naphtha 11A	143–160	95	4	1
Naphtha 11	143–167	97	2	1
Naphtha 10	158–170	97	2	1
Naphtha 4	160–180	92	7	1
Naphtha 3	180–200	99	—	1
Creosote oil 6	200–240	99	—	1

In the case of Creosote oil 6 only 1 maggot out of 144 (total of three tests) was not killed and it appeared to have escaped contact with the mixture. The result given by Naphtha A is unexpected, although this is the average of three tests which all gave similar results. The other fractions all show up well, but the 100 per cent. efficacy mark has not yet been reached, while 5 per cent. Cresol is probably too high for practical purposes.

VI. ADDITION OF ACID.

At this stage indications had been obtained from "feeler" tests that the addition of an acid, which would neutralise the medium around the maggots, may assist a contact larvicide and consequently acids were incorporated in the mixtures in subsequent tests. It was considered desirable to use an acid which is normally in the solid phase and will dissolve in the alcohol-tar oil mixture, in order that it should remain on the surface of the wound when the liquid has evaporated and maintain an acid reaction there. A list of possible acids was considered. Many of them would be irritating and others appeared to have other disadvantages. Eventually tartaric, citric and boric acid were selected for testing.

At the same time an oil was incorporated to prevent too hard and brittle a crust from forming on the wound. It was found that only a limited quantity of oil would mix with the alcohol-tar oil mixture. The only oil which mixes with the alcohol appeared to be castor oil, while all other vegetable, animal and mineral oils tried would not, although they readily mixed with the tar oils. The larger the quantity of oil incorporated the less alcohol had to be used in order to obtain a clear, homogeneous mixture. The addition of cresol improved matters, while the acids again had the opposite effect. It was, therefore, now a matter of balancing the ingredients in such a way that the desired result would be obtained. Since vegetable and animal oils definitely reduced the larvicidal efficacy of the mixture, only mineral oils could be used and further tests were made with liquid paraffin of S.G. 0·85. It was found that too large a proportion of mineral oil promoted the growth of bacteria and the formation of pus in the wound, while 10-15 per cent. of oil was sufficient to have the desired soothing and softening effect. The results obtained with some of the mixtures tested can best be presented in tabular form.

In these investigations some 700 tests were made, but it is unnecessary to record many of them, as they are of no direct interest.

TABLE 6.

96 Per Cent. Alcohol.	Tar Fraction.	Liquid Paraffin.	Cresol.	Acid.	L.	P.	F.
55	Naphtha 11 : 35.....	10	2·5	Citric 2...	97	2	1
55	Naphtha 11 : 35.....	10	2·5	Tartaric 2.	100	—	—
55	Naphtha 11 : 35.....	10	2·5	Tartaric 3.	100	—	—
55	Naphtha 11 : 35.....	10	2·5	Tartaric 4.	100	—	—
55	Naphtha 11 : 35.....	10	2·5	Tartaric 5.	100	—	—
55	Naphtha 11 : 35.....	10	2·5	Boric 2...	91	7	2
55	Naphtha 11 : 35.....	10	2·5	Boric 3...	100	—	—
55	Creosote oil 6 : 35...	10	2·5	Citric 2...	93	6	1
55	Creosote oil 6 : 35...	10	2·5	Tartaric 2.	100	—	—
55	Creosote oil 6 : 35...	10	2·5	Tartaric 3.	100	—	—
55	Creosote oil 6 : 35...	10	2·5	Tartaric 4.	100	—	—
55	Creosote oil 6 : 35...	10	2·5	Tartaric 5.	100	—	—
55	Creosote oil 6 : 35...	10	2·5	Boric 3...	100	—	—
50	Creosote oil 6 : 30...	20	3	Boric 3...	97	2	1
50	Creosote oil 6 : 30...	20	3	Tartaric 3.	100	—	—
40	Creosote oil 6 : 40...	17	3	Tartaric 3.	100	—	—

While tartaric acid in concentrations of 2, 3, 4 and 5 per cent. reduces the pH of the strike wound, which is usually about 8·4 before treatment, to 4·5, 4·3, 3·3 and 2·6 respectively, 2 per cent. citric acid produces a pH of approximately 4 and 3 per cent. boric acid only a pH of about 6·8. Apparently it is not the pH alone that is important. The results obtained with tartaric acid were consistently satisfactory, also in other tests not recorded here, and it was therefore finally selected as suitable.

Owing to the international complications it soon became difficult to obtain tartaric acid and its price rose above reasonable limits. Another readily available acid had, therefore, to be found and renewed attempts were made to incorporate wattle extract into the mixture. The tannates contained in this substance readily dissolve in alcohol, but all attempts to obtain a stable mixture failed owing to the fact that such a solution appears to be incompatible with some ingredient in the tar fractions. Eventually sulphuric acid was tried. Two difficulties had been expected, viz., that this acid would tend to cause polymerisation of parts of the tar fractions and that it might affect metal containers in which such a dressing would have to be supplied. Besides, there was the fact that such an acid would probably not remain on the surface of the wound as long as tartaric acid which would crystallise out there. At first diluted sulphuric acid was used in order to prevent polymerisation but a homogeneous mixture could not be made unless the proportion of alcohol was reduced below effective limits. However, if the concentrated acid was first diluted in the 96 per cent. alcohol it proved to have no further polymerising effect on the tar fractions. Moreover, a homogeneous mixture obtained in this way does not affect metal. This is probably due to the small quantity of water contained in the mixture being physically bound in such a way that the acid is unable to dissociate in it and therefore it remains inactive. It is again a matter of balancing the ingredients, with the tar fraction, liquid paraffin and cresol on one side and the alcohol with its water content and the acid on the other. Within certain narow limits the proportions of the various ingredients can be varied. The results obtained with such mixtures are given in Table 7.

TABLE 7.

96 Per Cent. Alcohol.	Tar Fraction.	Liquid Paraffin.	Cresol.	H_2SO_4.	L.	P.	F.
42	Naphtha 10 : 40.......	15	2·5	0·5	100	—	—
40	Creosote oil 6 : 44·5...	12·5	2·5	0·5	100	—	—
72·5	Creosote oil 6 : 25.....	—	2	0·5	100	—	—
72·5	Creosote oil 6 : 20.....	5	2	0·5	100	—	—
25	Creosote oil 6 : 72·5...	—	2	0·5	86	8	6
40	Creosote oil 6 : 42.....	15	2·75	0·25	100	—	—
43	Creosote oil 6 : 43.....	11·25	2·5	0·25	100	—	—

A concentration of 0.·25 per cent sulphuric acid produces a pH of about 4·5 in the strike wound immediately after treatment. A few hours later the pH has usually risen to 5·5-6, but then the surface of the wound becomes dry and, if it is moistened with distilled water 24 hours after treatment a pH of approximately 7 is usually obtained.

The mixture mentioned last in Table 7 was finally selected as the most suitable. Under field conditions it has given excellent results both against sheep blowflies and cattle screw worm (*Chrysomyia bezziana*). Restrikes were rare and limited to cases of continued scouring or soiling by urine and fighting rams.

VII. EFFECTS ON THE SKIN.

The testing of larvicidal efficacy on artificial strikes enables one at the same time to observe the effects of such mixtures on the sheep. The sheep's skin is very delicate and frequently the undamaged skin next to a strike area is affected more severely by an irritant mixture than is the damaged skin in the strike area.

The neutral naphthas and creosote oil do not damage the healthy skin, nor does the addition of cresols, even up to 5 per cent., cause undue irritation. With over 3 per cent. cresol the skin will show a slight reddening after 24-48 hours, but this is transitory. Strong alcohol usually produces a slight hyperaemia of short duration, not followed by any other changes. The addition of cresol to alcohol has no more marked effect, but the further addition of 0·25 per cent. sulphuric acid enhances the hyperaemia and in some cases a very thin, light-brown scab is formed and cast off. Tartaric acid does not produce any such effect. Just as the alcohol-tar distillate combination has strong penetrating properties as far as the maggots are concerned, it also appears to have such properties in relation to the superficial layers of the sheep's skin. The damaged skin in the strike area does not appear to suffer, since the result of treatment in this area is not worse than that obtained by washing the area with water after mechanical removal of the maggots. In both cases the area becomes dry, the surface hardens and forms a thin scab which is cast off after about a week. The healthy skin, treated with the same mixture, shows moderate hyperaemia and turns dark-brown within 24 hours. The surface becomes dry and unpliable and after about a week a thin scab is cast off, leaving healthy skin below. The addition of liquid paraffin reduces the hardening of the surface. The complete mixture produces the same results and, although this effect is superficial, it is the one remaining undesirable quality of this dressing. However, of a

number of farmers who have tested the dressing not one has complained about or even remarked on this effect. Other species of animals which have been treated show little or no skin reaction. A number of young white pigs, about 6 weeks old and badly affected with sarcoptic mange, were thoroughly sprayed all over. They showed a slight reddening of the skin, but this soon disappeared without any after-effects and the mange was cured by two applications. In cattle there was no skin reaction, not even in the ears when treatment was applied against ear-ticks. On the human skin the dressing has no adverse effect. The creosote oil (B.P. 200-240° C.) was selected as being slightly less irritant to the sheep's healthy skin than the light naphthas, while equal proportions of creosote oil and alcohol appeared to be better than a higher proportion of alcohol. The quantity of liquid paraffin that can be incorporated is limited by the proportions of alcohol and creosote oil.

VIII. General Remarks.

The cost of the sulphuric acid (commercial quality) is very low, but it remains to be seen whether it may not be preferable to replace it by tartaric acid in order to reduce irritation and to give better protection. As stated above, it may further prove desirable to incorporate a suitable stomach larvicide in order to extend protection against restrikes, but under present conditions this is not feasible without a great increase in costs.

As a matter of interest it may be mentioned that butyl alcohol does not produce the results obtained with ethyl alcohol and that combinations of pyrethrins in oil, naphthalene and paradichlorbenzene respectively with alcohol and acid gave as unsatisfactory results as these insecticides had previously given in other vehicles.

Summary.

An attempt was made to prepare a blowfly dressing which would be rapidly lethal to the maggots and cause the strike wounds to become unattractive to blowflies and to dry and heal quickly.

In vitro tests of contact larvicides on blowfly maggots were found to be unreliable. Tests were, therefore, made on artificial strikes.

The larvicidal efficacy of neutral tar oils is greatly increased by the addition of alcohol.

The further addition of a suitable acid increases larvicidal efficacy, makes wounds unattractive and promotes rapid healing.

A dressing is described which has given good results in field tests and which is also effective for treatment against screw-worm in cattle. The dressing readily kills ticks on animals and has given good results in a few cases of sarcoptic mange.

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The writer is greatly indebted to Dr. T. Jorden and Mr. C. v. d. M. Brink, of the chemical laboratory of Iscor (South African Iron and Steel Industrial Corporation, Pretoria), who prepared and supplied the various tar distillates used in these investigations, as well as to Mr. P. A. Cilliers,

technical assistant, who carried out large-scale field tests on sheep. A number of farmers in various parts of the Union also carried out tests on sheep and cattle and made very useful remarks on the mixtures submitted to them.

Addendum.

When production of the blowfly remedy was started on a large scale (over 20,000 gallons were sold during the first seven months) it soon became evident that a sufficient quantity of neutral creosote oil would not be available in the Union if the demand increased according to the indications. Moreover, the extraction of the tar bases and naphthalene, as carried out under present conditions in the large-scale production of the oil, was incomplete, leaving varying amounts of both, with the result that the remedy sometimes caused undesirable irritation.

Tests were therefore made with mixtures of benzol and creosote oil, instead of the latter alone, and finally a mixture of equal parts of these substances was decided on. This would allow the production of the remedy to be doubled with the same quantity of creosote oil and reduce the irritant ingredients in the latter to one half in the mixture.

The addition of benzol further allows the addition of more mineral oil as well, although, as noted previously, this should not be pushed too far. Supplies of mineral oil presented a further difficulty, as liquid paraffin became practically unobtainable. Various mineral oils were tested and satisfactory results were eventually obtained with a mixture of one part second grade motor oil (S.A.E. 60) and two parts of a light fuel oil (C.1. Fuel).

The varying amounts of tar bases remaining in the creosote oil made it necessary to adjust the amount of sulphuric acid incorporated in the mixture. Tests on this point indicated that the original quantity of neutral creosote oil used in previous experiments must have contained some tar bases and that 0·15 per cent. commercial sulphuric acid in the final mixture is sufficient to give a pH of 4·3—4·5 if the oil contains no bases. This degree of acidity is satisfactory and should be aimed at by adding the required quantity of acid according to the percentage of bases present.

The mixture as now made has the following composition:—

96% alcohol	40% volume
Mineral oil (S.A.E. 60)	5·0
Mineral oil (C.1. Fuel)	10·0
Cresol	2·5
Sulphuric acid	0·15
Benzol	21·175
Neutral creosote oil (B.P. 200—240° C.) ...	21·175

In practice the creosote oil is washed free of cresols only so far as to leave about 12 per cent., which gives the correct proportion in the final mixture, and therefore no cresol is added.

The efficacy of the mixture against maggots and ticks remains as high as before and no apparent pain is caused by its applications to strike wounds.

As previously noted such mixtures are irritant to the healthy skin of sheep, although they cause much less irritation to the affected parts in the strike area and none at all to the skins of cattle, pigs, horses and dogs. In the course of these additional tests it was noted that the same mixture did not affect all sheep equally and further observations indicated that it is the amount of wool grease which causes the variation, sheep with a relatively dry fleece being less affected than those with much yolk. Since the remedy dissolves wool grease it is very probable that the latter promotes absorption and in this way produces the particular sensitiveness of the sheep's skin.

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Ticks in the South African Zoological Survey Collection. Part II.

By GERTRUD THEILER, Section of Parasitology, Onderstepoort.

AMBLYOMMA SYLVATICUM, de Geer, 1778.

Synonym: A. latum Koch 1844.*

The Lesser (South African) Tortoise Tick.

Male (Figs. 1, 2 and 3.)

A SMALL ornate tick; up to 5 mm. × 4 mm.; very broadly oval, widest at about the middle; *Conscutum* somewhat convex. Cervical grooves, short, deep and narrow and slightly curved with convexity external. Marginal grooves absent, represented by a row of evenly spaced, large punctations commencing some distance behind the eyes and ending at the first festoon. A background of small, shallow, somewhat unevenly distributed punctations present on the whole conscutum; a few large coarse punctations on the shoulders, the lateral and posterior parts, the festoons and marginal ridge. *Eyes,* small, dark, hemispherical, deeply orbited; far forward, on a level with the cervical groove. Festoons well marked. The ornamentation is limited to a narrow pale strip, which may show an iridescent sheen, edging the "marginal groove" and extending on to the first two festoons. The outline of the female scutum may be quite pronounced.

Basis capituli.—Subtriangular, with lateral margins convex and postero-lateral corners also rounded, deep brown in colour and pale at the base of the whitish sheath. The *subcollare* (of Schulze 1935) is enamelled. Palps relatively broad, constricted at base of article 2; long white hairs present; article 2 twice as long as article 3; article 3 broader than long with anterior extremity rounded; white enamelling edging posterior, internal and anterior margin of article 2; internal and anterior margin of article 3. Ventrally the anterior point of article 1 is also enamelled. Hypostome 4/4, teeth in anterior 1/3; ventrally the basis capituli is subcircular.

Ventral surface lighter in colour than dorsal. Genital pore opposite coxae II.

Legs dark brown, with narrow white enamelled distal annulations, and a dorsal enamelled strip on all segments, except on the tarsus, which shows no enamelling whatsoever; pale hairs present. Tarsal termination as in Fig. 3, a and b. Coxae i-iii, a pair of short rounded spurs; on Coxa iv the inner spur usually much smaller than the outer and quite inconspicuous.

* For further synonymy see Bequaert 1932.

Female (Fig. 4).

A medium sized ornate tick; when engorged up to 14 mm. *Scutum:* heartshaped, postero-lateral margin sinuous; cervical grooves, deep and narrow, curved to form an inverted S. No lateral grooves. A background of medium-sized somewhat unevenly distributed punctations; a few large punctations on the shoulders, and anteriorly on the central field. *Eyes* small, dark, hemispherical, deeply orbited. The pale ornamentation is widespread, leaving a fairly wide cervical stripe. A dark marginal band joins the large ocular spot anteriorly with the cervical stripe, and posteriorly with the limiting spot; frontal spot small, irregular.

Basis capituli roughly triangular with lateral margin convex, and postero-lateral corners also rounded; deep brown in colour, pale towards the base of the sheath (Robinson figures it as having a light patch between the porose areas). Areae porosae oval, diverging anteriorly; distance apart not quite twice the diameter. In the two females available it is difficult to see whether the *subcollare* is enamelled as in the male or not; palps and hypostome as in the male (Robinson gives article 2 as three times as long as 3).

Ventral surface.—Legs, coxae and tarsi as in male, except that the spurs on coxa iv are more equal in size.

Nymph (Fig 5). Length 2 mm. to 3 mm.

Scutum ornate; slightly broader than long, postero-lateral margin straight, or but slightly sinuous, posterior angle broad; cervical grooves deep and strongly curved, with convexity external, the posterior tip may bend slightly outwards, giving an S shaped groove (as described for the female). Punctations, large, deep, fairly far apart, fairly evenly distributed. *Eyes* small, dark, hemispherical, deeply orbited. The ornamentation is very striking, a broad enamelled strip present in the lateral field extending almost to the anterior edge of the collar; it is interrupted by a dark frontal spot; the anterior portion of the central field is also enamelled, the enamelling edging the emargination right up to and including the small knob on the shoulder. The area around the eye is darker than the rest of the scutum.

Basis capituli triangular with rounded corners as in the male and the female. The enamelling occupies the central area and is roughly hour-glass shaped. Palps as in the male, with article 2, however, not quite twice article 3.

Legs as in the male; coxae i and ii with a pair of short rounded spurs; coxae iii and iv with external spur only (Bedford and Hewitt give coxa iii with two spurs, and coxa iv with a trace of an inner spur).

Larva (Figs. 6 and 7). 1·1 mm. × ·8mm.

Subcircular widest in posterior portion; convex. *Scutum:* much shorter than broad (1:1·75). Cervical grooves short, narrow, slightly curved. *Eyes* relatively large, hemispherical, orbited; about midway. Colour of scutum light brown, with deeper pigmentation round the eyes as in the nymph and adults; no light patches or enamelled ornamentation could be seen in the specimens examined; *Basis capituli* subcircular, wider than long. Palps broad, article 3 about as long as 2 (long white hairs?); article 3 with a marked ridge below article 4 on the ventral surface. Hypostome 2/2 of 5 teeth, on anterior 1/3.

Legs.—Coxa i with internal spur only; coxae ii and iii with a broad flat spur towards the middle of the posterior margin.

Host.

The Onderstepoort collection contains the following lots off the tortoise *Chersinella schönlandi*, from Namaqualand: 5 males; 1 female; 1 female + larvae; and one lot of males and nymphae off the tortoise, *Chersine angulata* from Essendene, Eastern Province (this is the batch described by Bedford and Hewitt 1925).

Bedford and Hewitt also list it from *Chersine angulata* from Malmesbury, Western Province; from Port Elizabeth, Eastern Province; and from a mole snake, *Pseudaspis cana* from Port Elizabeth.

L. E. Robinson in the Monograph lists it from the Cape of Good Hope; off a tortoise Cape of Good Hope, and from Kaffraria (Eastern Province); and 1 female off a Virginian deer in the Zoological Gardens, Hamburg.

Warburton (1927), lists three tubes of specimens in the Vienna Museum as from Natal and " Cape Zelabor " (I have not been able to find this Cape on any map of South Africa) and gives the one host as one of the common Cape tortoises—*Homopus areolatus*, and the other host as *Tityus lineatus*. (An American scorpion !—undoubtedly another case of interchange of labels).

De Geer's original specimen was collected off a tortoise by Sparmann in his travels at the Cape.

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Fig. 1.

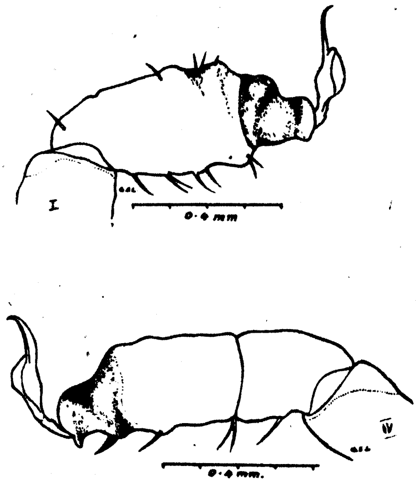

Fig. 3.

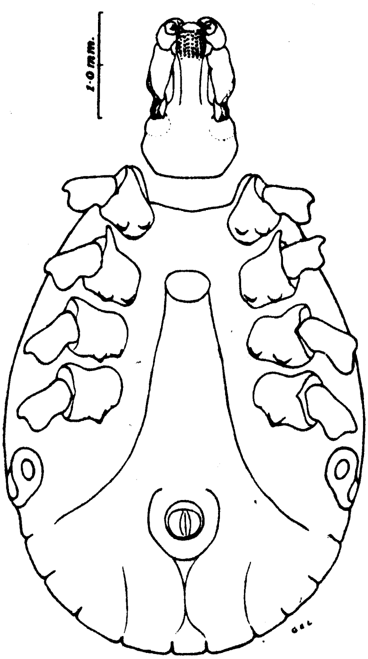

Fig. 2.

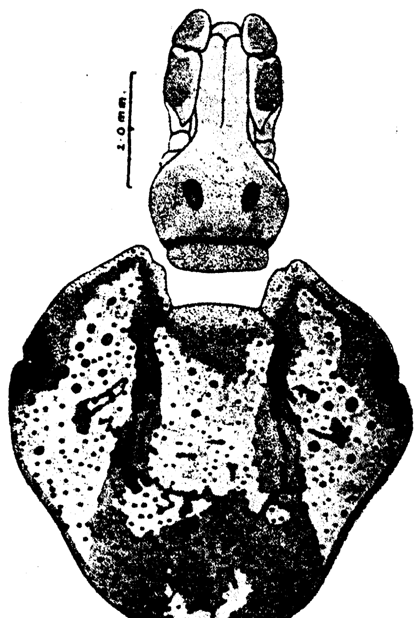

Fig. 4.

Fig. 1.—Male Dorsal View. Bedford del.

Fig. 2.—Male Ventral View. G. E. Laurence del.

Fig. 3.—Tarsus I and Tarsus IV. G. E. Laurence del.

Fig. 4.—Female Dorsal View. G. E. Laurence del.

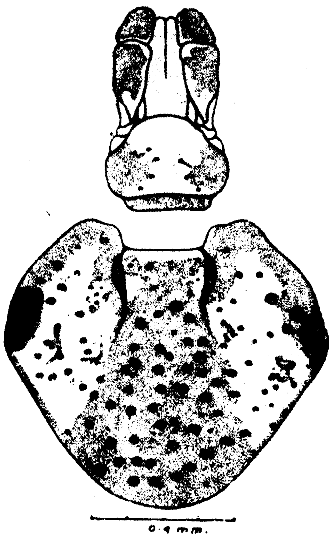

Fig. 5.

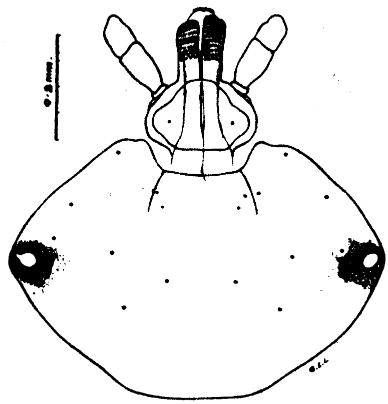

Fig. 6.

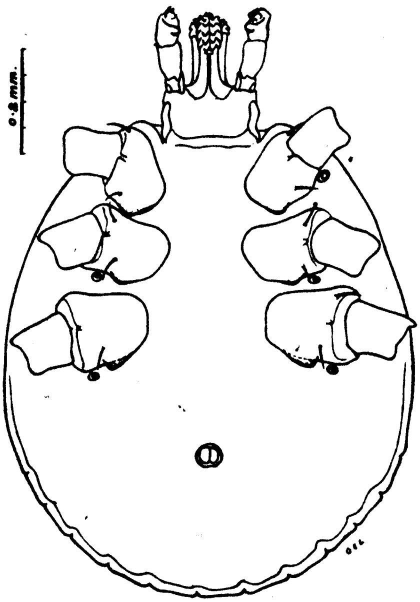

Fig. 7.

Fig. 5.—Nymph Dorsal View. G. E. Laurence del

Fig. 6.—Larva Dorsal View. G. E. Laurence del.

Fig. 7.—Larva Ventral View. G. E. Laurence del.

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Studies on the Alimentary Tract of Merino Sheep in South Africa VII.—Fermentation in the Forestomachs of Sheep.

By J. I. QUIN, Section of Physiology, Onderstepoort.

I. Introduction.

THE nutrition of ruminant animals has within recent years developed into a matter of very special significance. This was due primarily to the realization of the economic importance of various products derived from these animals and in consequence of which the world demand for them has steadily increased. As it was further realised that the ruminant body was peculiarly adapted for the transformation of bulky, inexpensive plant material into such valuable products as meat, milk, and wool, a vast programme of research work has been undertaken on the nutritional requirem nts of various classes of ruminants relative to type and level of production. Moreover the appearance of a wide variety of deficiency states in ruminants acted as a further incentive for more detailed investigations of their nutrition. As a result of all these considerations much knowledge has recently been gained on this particular subject. Based on a wide range of metabolism studies, one of the most important findings in all this work consisted in demonstrating the basic nature and nutritional significance of so-called balanced rations. Much of this information was obtained by the well-known methods of trial and error usually comprised of a correlation between:—

(*a*) the diet fed,

(*b*) the digestibility and utilization of individual nutrients, and

(*c*) body response.

By these methods the significance of the balanced diet, including the relative importance of a wide variety of individual factors, has been clearly established in ruminant nutrition. This has resulted in a decided improvement in production as well as in the successful control of a number of deficiency diseases.

In spite of this newer knowledge, the nutrition of ruminants nevertheless still presents a variety of unsolved problems. This is due, at least in part, to the intricacies of digestion, the physiology of which is less clearly understood in the ruminant than in any of the other domesticated mammals. This, indeed, constitutes an important limiting factor as far as a complete understanding of the nutritional problem in these animals is concerned. In answer to the question why the processes of ruminant digestion should be considered as being more involved than those in other classes af animals, it should be

emphasized that this is due primarily to the presence of the various fore-stomachs in the digestive system. Seeing that processes whereby food materials are broken down in these compartments are so completely different from those usually associated with the enzyme systems operating in the rest of the digestive tract, it is essential that the peculiarities and relative significance of these two distinct phases of digestion be studied separately. It is to be expected that more detailed investigations along these lines would increase not only our knowledge concerning the basic principles of ruminant nutrition, but at the same time help to elucidate the aetiology of the large variety of digestive disturbances to which ruminants are subject.

In considering the normal conditions present in the forestomachs, it is to be noted that while there is complete absence of digestive secretions from their walls, these organs provide an exceptionally favourable environment for the establishment of a wide variety of bacteria, infusoria, and other micro-organisms which usually contaminate the food supply. It is due largely to the maintenance of optimal conditions in the forestomachs concerning food supply, moisture, temperature, hydrogen ion concentration, oxygen and carbon dioxide tensions, and removal of metabolites, that various groups of micro-organisms are capable of establishing themselves and even of forming such very dense populations in the ruminal mass.

Through the researches of Czepa and Stigler, Wester, Trautmann, Krzywanek, Dukes, Schalk and Amadon, Phillipson, Quín and van der Wath and various other workers, much information has been gained within recent years concerning the physical and mechanical aspects of rumen physiology. Thus the process of regurgitation during rumination and the factors associated with the motility of the forestomachs have been fairly fully elucidated.

In respect to the chemical changes undergone by the food mass in its passage through the forestomachs and the relation that these changes bear to the other phases of digestion, our knowledge is as yet far from complete. It is known, however, that through the presence of various types of bacteria in them, the forestomachs are mainly associated with the disintegration of cellulose and other fibrous food constituents. Likewise acid fermentation of carbohydrates has been established. According to the findings of McElroy and others, the rumen forms the seat of an important vitamin B synthesis. Concerning the significance of the different organisms, Mangold, mainly through the work of Ferber, considers the ruminal infusoria as playing an important role in the biological ennoblement (biologische Veredlung) of plant proteins which, after their transformation into infusorial protein, are rendered more easily available to the animal body. Becker, Schulz and Emmerson on the other hand regard the infusoria merely as commensals and thus without any specific importance in ruminant digestion. Van der Wath, in a recent publication, likewise concludes that ruminal infusoria are not essential, seeing that digestion is well maintained in sheep from which these organisms have been banished. Kleine, in referring to all the micro-organisms in the forestomachs as " Pansen Plankton " (ruminal plancton), considers this as constituting an important item in the diet of the host animal.

While large numbers of micro-organisms are constantly passed out with the ingesta from the forestomachs to undergo disintegration and absorption in the rest of the digestive tract, there are as yet no data available to indicate the exact significance of this in facilitating digestion and thereby of

improving the nutritional state of the animal. All food materials swallowed into the forestomachs are exposed to the action of a densely populated, mixed culture of micro-organisms which has to maintain itself in the face of constant drainage. In contrast to the well-known hydrolytic processes usually associated with digestion, the changes undergone by the food during its passage through the forestomachs are indeed far more complex, seeing that these changes are determined by the constitution and metabolic activity of the microflora present. Accompaying the disintegration of various food materials during this phase of digestion, new compounds are constantly being synthesised in the forestomachs. Little is as yet known about the nature and significance of these products, although the formation of vitamin B complex referred to above serves as an example. Likewise the utilization of such nitrogenous compounds as urea and various ammonium salts in the production of new proteins within the rumen, may be quoted as further evidence of the synthetic powers of the ruminal flora. (Kleine, Schmid and Studt, Harris and Mitchell.)

From these considerations it may be concluded that ruminant nutrition is at least partly dependent upon the products of bacterial metabolism which are rendered available for absorption. As a result of these processes in the forestomachs, the biological value of the food consumed may be altered in such a way as to lead either to its improvement or to its deterioration depending upon the type and density of the microflora. In order to achieve and to maintain optimal conditions within the forestomachs in respect to both the nature of the processes and the speed at which they occur, it is essential, therefore, to stabilize as far as possible this mixed population of micro-organisms by constant satisfaction of the various requirements. In this respect the adequacy or otherwise of their food supply, including that of minerals and other vital factors is of no less importance than that applying to any mixture of micro-organisms under artificial cultivation. A deficiency of any one essential nutrient should, therefore, result in some form of disturbance in the microflora in which either the population as a whole or some of its individual members are subjected to a depression. Should such a disturbance be sufficiently severe, it is obvious that the various processes normally occurring in the forestomachs might be replaced by a series of pathological conditions expressed in symptoms such as atony, stasis, hoven, or diarrhoea.

Concerning the infusorial count in the forestomachs, Ferber in his studies has been able to correlate this with the level of protein intake as well as with the metabolic changes associated with growth and lactation. More recently van der Wath, in a study on bacterial counts in rumen ingesta, demonstrated wide fluctuations in his data depending upon the intake level of proteins and carbohydrates.

From information thusfar available there is evidence to suggest, therefore, that a continued and complete satisfaction of the nutritional demands of the mixed ruminal flora may in itself form an essential prerequisite for the adequate nutrition of the host animal. How a normal flora is constituted, what its food requirements are, and of what specific value it is in ruminant nutrition, all form problems yet to be elucidated. Seeing that fermentation constitutes one of the most characteristic features of ruminal activity, a series of investigations were initiated primarily with the object of studying the nature of the fermentation process, and of the factors influencing it, and finally in correlating if possible, these data with the pathogenesis of bloating.

II. Methods Employed on Fistula Sheep.

Using adult merino sheep with permanent ruminal fistulae, Quin, van der Wath and Myburgh in a previous communication described a method for the continuous recording of the amount of gas generated within the rumen. This was achieved by connecting the rumen through its fistula tube to a large graduated water manometer. By equalizing the water level in the two limbs of the manometer the volume of gas could be read off directly at constant atmospheric pressure. From data collected it was soon evident, however, that there were several drawbacks to this method. Firstly, it was found that the gas volume recorded was subject to a double fluctuation which was due either to a change in the rate of fermentation or to a rise or fall in intra-ruminal pressure coinciding with the movements of the forestomachs. Where the gas volumes were being recorded for periods longer than 10 minutes, the effect of ruminal movements on gas pressure could, however, be clearly differentiated from that due to a change in fermentation rate. A further difficulty arose from the fact that filling of the rumen with rapidly fermentable materials frequently led to frothing up of the ingesta followed by blocking of the fistula tube. As a rule this could be overcome by limiting the amount of test material introduced into the rumen.

Considering the gas production as an index of the fermentation processes in the rumen, an attempt was made in this study to correlate the volume and rate at which gas was generated with various food materials, and with conditions prevailing in the forestomachs.

III. Influence of Diet on Gas Production in the Rumen.

The routine procedure in these tests was to place two or more fistula sheep on a specified diet for several weeks. Animals were kept in separate pens so as to allow for individual feeding and watering. Both food and water consumption were determined daily. During the recording of gas volumes, animals were placed in a specially constructed crush pen, thereby restricting movement during the period in which the manometer was connected to the fistula tube. Provision was made for feeding and watering when required during these periods. Gas volumes were continuously recorded every 5 minutes for 15-30 minutes, usually in the early morning and immediately prior to the consumption of the test meal, the recording being continued for several hours afterwards.

Preliminary observations conducted on sheep in the crush-pen disclosed a frequently repeated eructation of gas through the oesophagus, usually within a few minutes after the consumption of food such as green lucerne. At times this eructation was distinctly audible while at other times it was discernible merely as a shallow retroperistaltic wave in the oesophageal region. This eructation of gas from the rumen was, however, stopped as soon as the manometer was connected to the rumen and a rise in the gas pressure prevented by the maintenance of a constant atmospheric pressure in the manometer. From these observations it was safe to conclude that all " free " gas was finding its way into the manometer instead of escaping under pressure through the oesophagus.

In Table 1 is recorded the results obtained by feeding a variety of test foods either to the same sheep or to different ones during the course of the experiment. In none of the animals examined was there any sign of gas production in the early morning before feeding, the only fluctuation in the manometer readings being that due to the ruminal movements. As shown in

TABLE 1.

Effect of Diet on Gas Production in Rumen.

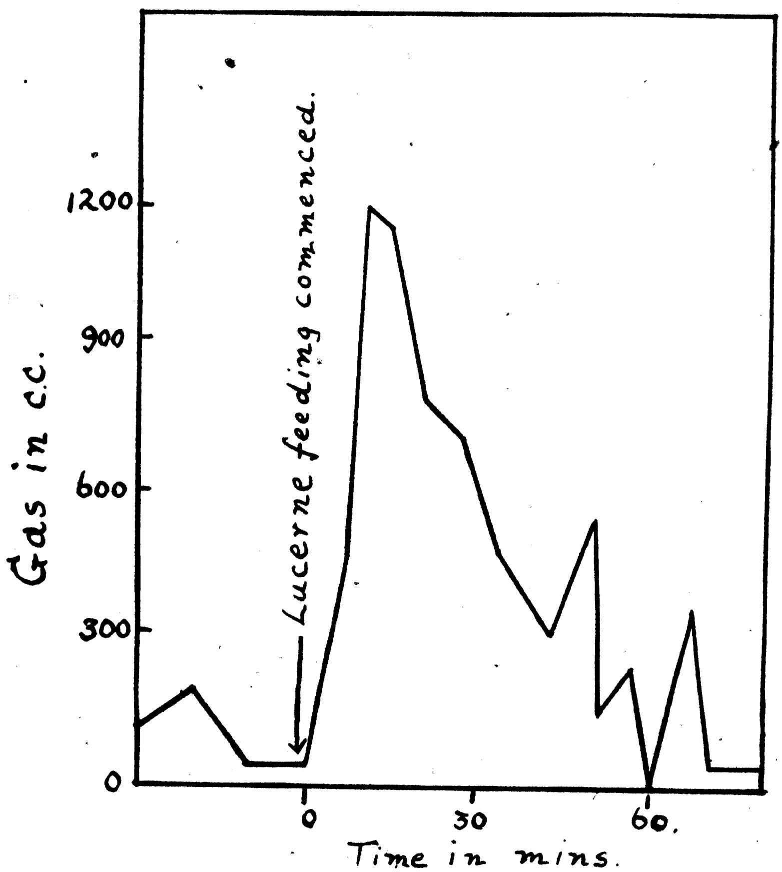
Sheep No.	Basic Ration.	Test Meal.	Gas Production (5 Minute Intervals), in c.c.																		Total Amount of Gas Produced in 90 Mins.
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
1	Green lucerne (3 Kg. daily)	Green lucerne 1·5 Kg. consumed in 45 mins.	420	300	520	850	760	640	580	600	600	410	400	480	450	450	340	500	440	325	9,065 c.c.
2	Green lucerne (3 Kg. daily)	Green lucerne 1·5 Kg. consumed in 55 mins.	200	310	350	360	360	350	500	200	360	120	220	140	200	80	120	50	180	50	4,550 c.c.
3	Wheat straw 600 gm. daily	Wheat straw 205 gm.	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0
4	Lucerne hay 450 gm. . .	Lucerne hay 200 gm. finely chopped and dosed through fistula	0	140	510	470	500	450	330	260	180	140	140	190	200	170	110	240	300	190	4,520 c.c.
5	Green lucerne (3 Kg. daily)	Wheat straw 200 gm. powdered and dosed through fistula	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0
6	Green lucerne (3 Kg. daily)	Maize samp 100 gm. finely powdered and dosed through fistula	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0
7	Green lucerne (3 Kg. daily)	Cane sugar 50 gm. dissolved in 250 c.c. water and dosed through fistula	0	100	540	550	780	470	350	500	290	390	440	230	60	90	60	40	90	50	5,030 c.c.
8	Green lucerne (3 Kg. daily)	Glucose 50 gm. in 200 c.c. water dosed through fistula	0	0	250	640	670	620	450	420	510	140	40	170	60	100	20	20	0	0	4,110 c.c.
9	Lucerne hay (1 kg. daily)	Glucose 50 gm. in 200 c.c. water dosed through fistula	0	0	260	520	900	800	570	220	130	180	40	10	20	70	40	0	0	0	3,760 c.c.
10	Poor quality grass hay 600 gm. daily	Glucose 50 gm. in 200 c.c. water dosed through fistula	0	240	250	105	100	250	190	190	140	180	200	140	80	80	160	160	20	20	2,505 c.c.
11	Poor quality grass hay 600 gm. daily	Glucose 50 gm. in 200 c.c. water dosed through fistula	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0
12	Poor quality grass hay 600 gm. daily	Glucose 50 gm. in 200 c.c. water dosed through fistula	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0
13	Maize meal 360 gm. and lucerne hay 300 gm. daily	Glucose 50 gm. in 200 c.c. water dosed through fistula *Sheep off feed for past 48 hours.*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0

* No gas produced over period of 90 minutes.

a previous communication (Quin and van der Wath, 1938) this varied from 100 mm. water pressure during the height of ruminal contraction down to 15 mm. below zero during the relaxation phase. The absence of active gas production in the early morning before feeding, affords evidence of the intermittency of the fermentation process, and of its dependence on fresh food in the forestomachs.

Discussion of Results Compiled in Table 1.

1. Sheep kept on a basic diet of lucerne only, which was fed either as green material or as dry hay, were able to cause rapid fermentation of weighed test meals of lucerne consumed in the early morning before routine feeding took place. The amount of gas produced over a period of 90 minutes varied from more than 9 litres (Sheep No. 1) to approximately 4·5 litres (Sheep Nos. 2 and 4). This difference in the total gas yield when identical test meals were fed to individual animals was a feature constantly noted and is to be ascribed solely to the conditions present within the forestomachs. In all these animals, nevertheless, fermentation was of a fulminating character since gas production invariably reached its peak within 30 minutes after the commencement of feeding to be followed by an equally rapid decline (see Curve 1).



CURVE No. 1.—Effect of Lucerne feeding on gas production in rumen of sheep.

2. The feeding either of wheat straw (Sheep No. 5) or of maize samp (Sheep No. 6) to animals accustomed to a diet of lucerne, failed to provoke any gas production within 90 minutes. Subsequent gas formation was at best very slow as compared with that noted on lucerne. This indicates that neither cellulose (wheat straw) nor starch (maize samp) is capable of the same rapid fermentation as undergone by certain constituents present in lucerne.

3. The dosing either of cane sugar (Sheep No. 7) or of glucose (Sheep Nos. 8 and 9) in amounts of 50 grams directly into the rumen of animals on a basic diet of lucerne caused prompt fermentation and gas evolution similar to that noted with lucerne itself. As similar fermentation was obtained with juice expressed from fresh green lucerne or with a watery extract from lucerne hay, it was evident that the sugar in the lucerne was the factor responsible for the very rapid fermentation.

4. In animals on a basic ration of poor quality veld grass hay, the introduction of test doses of glucose into the rumen provoked either no fermentation as in sheep Nos. 11 and 12 or a small gas yield of 2·5 litres produced over an extended period and without any sign of a " peak " during the first 30 minutes (sheep No. 10). In this connection it should be noted that sheep No. 10 displayed a particularly strong fermentation on a diet of lucerne and that even on a subsequent ration of poor veld hay its appetite was well maintained. In sheep Nos. 11 and 12, on the other hand glucose fermentation on a lucerne diet was less active while on a subsequent ration of poor veld hay their appetite rapidly declined and with it the power to ferment glucose. Likewise an animal on an adequate ration of maize meal and lucerne hay sheep No. 13), when off its feed for 48 hours, failed to ferment a test dose of glucose. These results indicate that when sheep are either completely starved or kept on a ration consisting of poor quality hay or straw, the power to ferment glucose in the forestomachs is readily suppressed in most animals. In the following section further details will be provided concerning the cause of this change in the fermentation process.

IV. In Vitro Fermentation Tests on Ruminal Ingesta.

A. *Technique.*

In order to obtain more data concerning the fermentation process in the forestomachs, a method was devised whereby small aliquots of freshly drawn ruminal ingesta were fermented *in vitro* and the gas yields determined. By these means several fermentation tests could be carried out simultaneously either on the same or on different samples of ingesta.

The routine procedure was to aspirate 100-300 ml. of the ruminal fluid from the fistula, usually before feeding in the morning when the consistency was more watery than at other times. After straining the material through muslin, amounts of 50 ml. each were poured into Erlenmeyer flasks of 250 ml. capacity and placed in a water bath the temperature of which was controlled at 39° C. Repeated readings previously carried out on the temperature in the rumen of fistula sheep were found to fluctuate round about 39° C. A moveable tray, operated by a small motor and capable of holding the flasks, was fitted inside the water bath thereby ensuring a constant to and fro movement of the material. The object of this was to imitate the mixing of the ingesta by the normal ruminal movements and to promote the escape of

gas from the fermenting mass. A suitable air-tight connection of each flask to an individual water manometer mounted on a wooden frame, allowed for direct readings to be taken on the gas volume at atmospheric pressure.

After a series of preliminary tests on the fermenting qualities of ruminal fluid, the standard method finally adopted consisted in the measurement of the rate of gas production recorded from one flask every 10 minutes over a period of 30 minutes immediately after the addition of 1 ml. 20 per cent. glucose solution (final concentration of 1 in 250 in ruminal fluid). This flask acted as control in a complement of 4 samples which as a rule were fermented simultaneously. Prior to the addition of the test material, the gas yield from the untreated ruminal fluid in each flask was likewise measured over one or more periods of 10 minutes each.

B. *Glucose Fermentation by Ruminal Ingesta.*

In a previous section evidence was submitted of the rapid fermentation undergone by glucose when introduced into the rumen of well-fed sheep as compared to ones on a poor diet. The same animals were used for supplying ruminal material in conducting the *in vitro* fermentation tests.

The following Table, 2, indicates the results obtained when 50 ml. amounts of ruminal fluid drawn from the same sheep on separate days, were fermented with 1 ml. 20 per cent. glucose, while the diet was kept constant at 1 kilogram lucerne hay daily.

TABLE 2.

Date.	Gas produced before adding Glucose (10 Minutes Period).	Gas produced after adding 1 ml., 20 Per cent. Glucose.			Total Gas production during 30 Minutes.
		1st—10 mins.	2nd—10 mins.	3rd—10 mins.	
1............	2·0 ml.	11·7	7·3	4·7	23·7
2............	0·9	8·8	8·2	2·8	19·8
3............	1·0	16·1	5·9	1·9	23·9

From the above table it will be noted that while there is practically no liberation of gas from the ruminal fluid drawn before feeding in the early morning, the addition of glucose to it provokes prompt fermentation and gas production which reaches its maximum within the first 10 minutes. During the following 20 minutes it rapidly reverts to a level only slightly higher than that noted before the addition of sugar. Moreover, there is a close correlation in the total amount of gas produced from ingesta collected on different dates. With constant shaking of the fluid during fermentation the total gas yield was found to fluctuate between 20 and 25 ml. in 30 minutes. In the absence of shaking movements on the other hand, the yield of gas decreased to less than half over the same period while the rate of fermentation became more prolonged. (Curve 2.)

Table 3 presents the results obtained in a comparative fermentation test using ruminal ingesta drawn before feeding from eight different fistula sheep. All animals were kept on a daily diet of 1 kilogram lucerne hay.

TABLE 3.

Glucose Fermentation in Ruminal Fluid from Different Sheep.

Sheep No.	Amount of Gas produced (ml.) before addition of Glucose (10 Minutes Period).	Amount of Gas produced after addition of Glucose.			Total Gas Production (30 Minutes).
		1st Period (10 Minutes).	2nd Period (10 Minutes).	3rd Period (10 Minutes).	
1	1·2	12·5	9·7	5·3	27·5
2	0·8	10·0	7·1	3·3	20·4
3	1·3	13·5	6·2	1·9	21·6
4	1·0	11·7	8·6	4·7	25·0
5	1·3	13·3	8·1	3·1	24·5
6	1·1	7·2	5·3	3·3	15·8
7	0·7	16·1	7·7	1·8	25·6
8	0·8	13·0	9·4	2·8	25·2

From the above results it can be concluded that with eight sheep kept on the same diet of lucerne hay, samples of their ruminal fluid show a close similarity in their power to ferment glucose, except in the case of sheep No. 6 in which the gas production is somewhat lower than in the other animals.

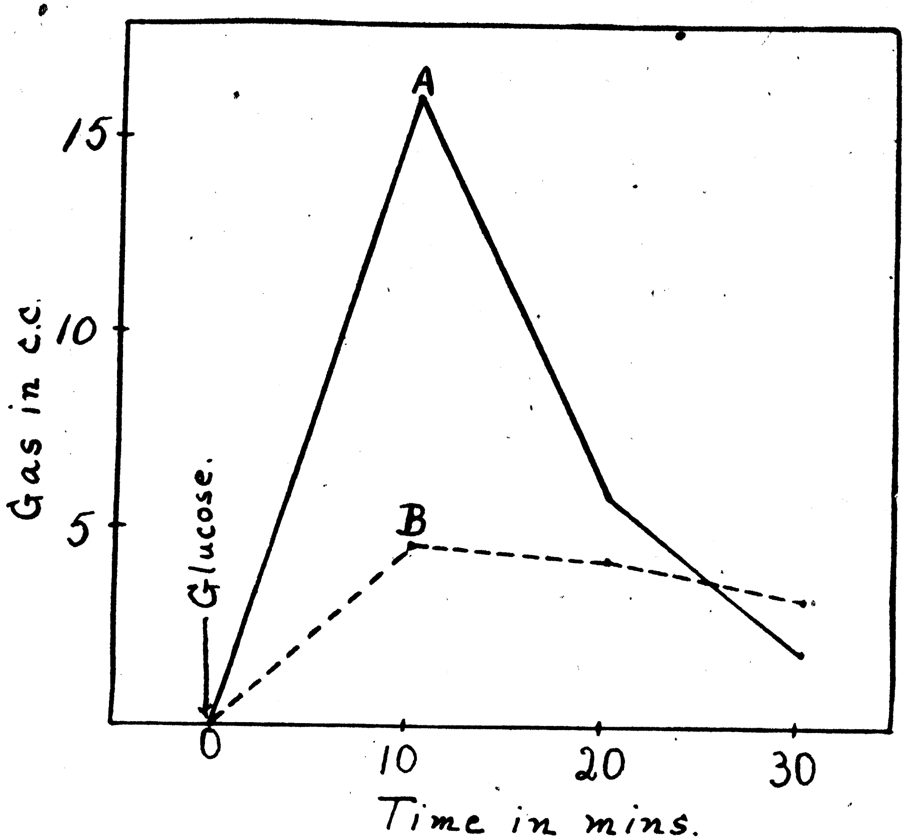
Influence of Glucose Concentration on Rate of Fermentation.

Seeing that glucose undergoes such rapid fermentation when brought into contact with rumen ingesta, a series of tests was conducted in order to ascertain how the rate of fermentation was influenced by varying the concentration of glucose present. This is illustrated in Table 4, in which rumen material from the same animal was used throughout the various fermentation tests.

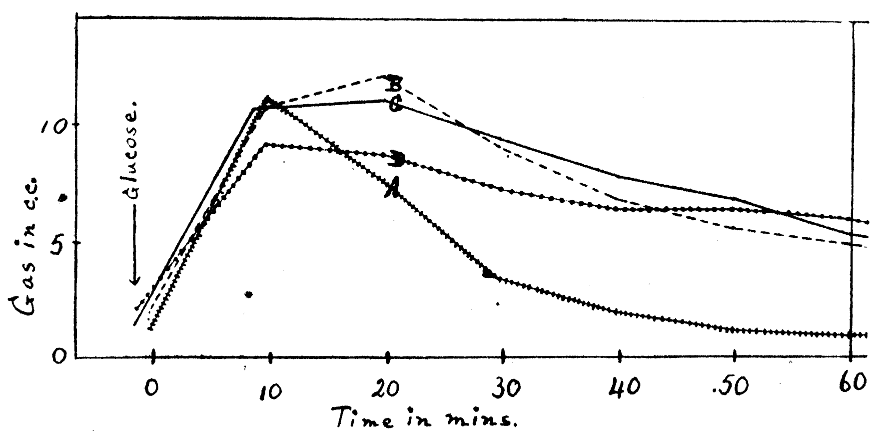
TABLE 4.

Influence of Glucose Concentration on Fermentation.

Glucose Concentration in 50 ml. Ruminal Fluid.	Gas Produced in 1st 10 Minutes.	Gas Produced in 2nd 10 Minutes.	Gas Produced in 3rd 10 Minutes.	Total Amount of Gas in 30 Minutes.
Per cent.				
0·1	4·4	1·2	0·2	5·8
0·2	7·0	3·1	0·5	10·6
0·3	11·3	3·8	0·5	15·6
0·4	12·7	7·2	1·1	21·0
0·8	12·5	11·7	5·6	29·8
1·2	12·2	12·8	7·8	32·2
1·6	12·0	12·5	8·0	32·5
5·0	10·9	12·0	9·0	31·9
10·0	10·6	10·9	8·7	30·2
15·0	9·1	8·6	7·2	24·9



CURVE No. 2.—Effect of shaking on glucose fermentation by rumen ingesta.
A=shaken. B=unshaken.



CURVE No. 3.—Influence of glucose concentration on fermentation by rumen ingesta.
A=0·4 per cent. glucose. B=5·0 per cent. glucose. C=10·0 per cent. glucose. D=15·0 per cent. glucose.

Data in the above table indicate that fermentation as judged by gas production, is closely related to the concentration of glucose up to a level of 0·4 per cent. With amounts greater than this the fermentation level taken over a 30 minute period, remains practically constant until a concentration of 15 per cent. glucose is reached when there are definite signs of depression in the fermentation rate. (Curve 3.)

Repeated addition of glucose at 30 minute intervals to the same rumen material was found to result in a rapid decline in the gas production which was clearly noticeable even when the second addition of glucose was made. This decline was characterized mainly by the disappearance of the initial high " peak " which was replaced by a lower and more even level of gas production when further amounts of glucose were added.

Likewise freshly drawn rumen material was found to lose its power of fermenting glucose on being continuously shaken for 3 hours in the warm water bath or when allowed to stand either at room temperature or at 0° C. for 24 hours. The addition either of glucose, molasses or of $\frac{N}{10}$ HCl to fresh rumen fluid did, however, preserve the fermenting quality of the fluid when this was subsequently tested with glucose after 24 hours standing. In Table 5 data are presented which illustrate this finding:—

TABLE 5.

Influence of Sugars and of Acid in Preserving the Fermenting Quality of Ruminal fluid.

Material added to 50 ml. fresh rumen fluid.	Total amount of gas produced when ingesta subsequently fermented with glucose after standing at room temperature for 24 hours.
1. Control flask	1·3 ml. gas.
2. Glucose 0·2 gram	20·5
3. Glucose 1·0 gram	0·7
4. Molasses 0·5 gram	21·7
5. Molasses 2·5 gram	Nil.
6. $\frac{N}{10}$ HCl 5 ml	11·9
7. $\frac{N}{10}$ HCl 10 ml	18·9
8. $\frac{N}{10}$ HCl 15 ml	19·0

From this table it is noted that whereas small amounts either of glucose or of molasses aid in preserving the fermenting power of ruminal fluid, larger concentrations definitely inhibit it. The addition of 15 ml. $\frac{N}{10}$ HCl likewise allowed normal glucose fermentation to take place 24 hours after the material had been drawn.

C. *Fermentability of Different Carbohydrates.*

In addition to the fermentation of glucose *in vitro*, a series of comparative tests was carried out on various other sugars and also on starch. The rumen ingesta used in these experiments was derived throughout from the same animal, while a control glucose fermentation test was included in every batch of samples fermented.

TABLE 6.

Fermentation of Different Carbohydrates by Rumen Ingesta (50 ml.).

Test Material.	Gas Formed 1st 10 Minutes.	Gas Formed 2nd 10 Minutes.	Gas Formed 3rd 10 Minutes.	Total Amount Gas formed in 30 Minutes.
	Ml.	Ml.	Ml.	Ml.
Glucose 0·2 gram	13·7	6·2	2·3	22·2
Fructose 0·2 gram	12·3	8·2	1·9	22·4
Sucrose 0·2 gram	11·0	4·0	1·8	16·8
Maltose 0·2 gram	3·1	3·1	3·2	9·4
Mannite 0·2 gram	2·6	2·8	3·2	8·6
Lactose 0·2 gram	2·2	1·6	1·3	5·1
Galactose 0·2 gram	2·1	1·0	1·2	4·3
Arabinose 0·2 gram	1·6	1·0	1·0	3·6
Xylose 0·2 gram	1·6	0·9	0·7	3·2
Rhamnose 0·2 gram	1·4	0·8	0·7	2·9
Raw starch (tapioca) 0·2 gram	1·1	0·9	0·4	2·4
Boiled starch (tapioca) 0·2 gram	1·0	1·0	0·7	2·7

The results in the above table indicate that while both glucose and fructose undergo an extremely rapid and identical type of fermentation, that of sucrose (cane sugar) is somewhat slower although still exhibiting a definite peak period within the first ten minutes. Maltose and mannite on the other hand are both fermented at a much slower speed, fermentation being prolonged and without evidence of any peak period within the first 30 minutes. Fermentation of both lactose and galactose is very feeble while that of arabinose, xylose and rhamnose is even less evident. Likewise starch, either raw or boiled, shows no signs of undergoing fermentative disintegration within 30 minutes. From the first hour onwards there is, however, a notable difference in the fermentation rate of the two starch samples. Thus soluble starch produced a total of 24·5 ml. gas within 4 hours as against a yield of 8·5 ml. only by the raw starch.

D. *Effect of Starvation on the Activity of the Ruminal Fluid.*

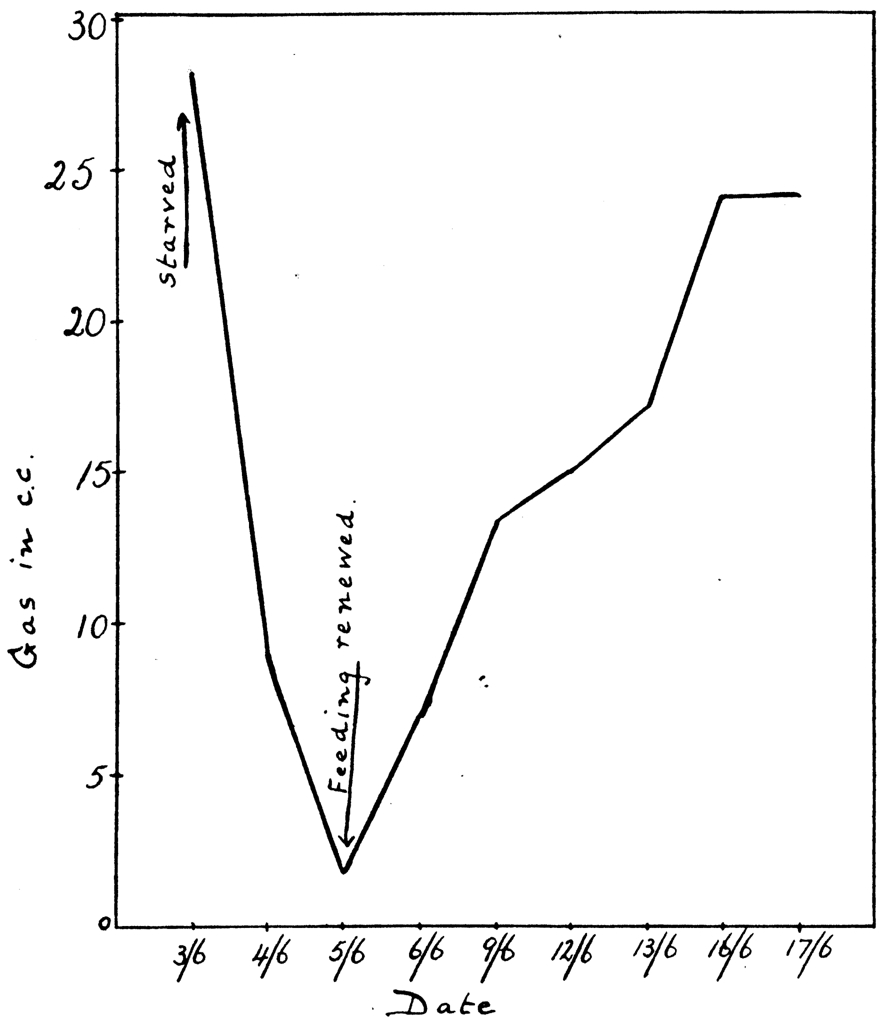
As indicated previously, starvation of a sheep for 48 hours resulted in a marked depression in the fermentation of glucose when dosed into the rumen. This observation was subsequently further investigated *in vitro* on rumen material withdrawn periodically from a fistula sheep undergoing total starvation and kept from water. The results of this test are set out in Table 7. The quantities of rumen material and glucose used were the same as those previously recorded.

TABLE 7.

Activity of Ruminal Fluid during Starvation.

Period of Starvation.	Amount of Gas Produced. 1st 10 Minutes.	2nd 10 Minutes.	3rd 10 Minutes.	Total for 30 Minutes.
17 hours	16·5 ml.	8·5	3·0	28·0 ml.
41 „	4·5	2·7	1·8	9·0 „
65 „	1·3	0·2	0·2	1·7 „

From this test it is evident that starvation causes a rapid and practically complete suppression in the fermenting power of ruminal ingesta. This is accompanied by increased wateriness of the material and the absence of any froth as usually noted in the ingesta of the normally fed animal. By restoring the animal to its full diet after having been starved for 65 hours, it was found that the normal daily consumption was reached only after the third day. Glucose fermentation on the other hand was even more profoundly influenced by starvation since the normal gas production was not restored until the tenth day after feeding had been resumed. (See Curve 4.) Results similar to the above were obtained in a subsequent experiment in which 5 sheep were starved for a period of 3 full days.



CURVE No. 4.—Effect of starvation of sheep on glucose fermentation by rumen ingesta.

E. Type of Diet in Relation to the Character of the Ruminal Fluid.

Using 7 fistula sheep, a long-term experiment was conducted with the object of ascertaining to what extent the ruminal fluid, as measured by its glucose fermenting properties, was influenced by various types of diet. This was divided into three distinct periods as follows:—

Period 1. (45 days).—All 7 sheep were on a basic ration of 1 kilogram lucerne hay daily. In addition sheep Nos. 1 and 2 received 100 grams cane sugar daily, while sheep Nos. 3 and 4 were each fed 200 grams crushed yellow maize daily.

Period 2. (97 days).—All 7 sheep were on a basic ration of 600-800 grams teff hay, the supplementary ration of sugar and maize being continued as in period 1.

Period 3. (60 days).—All 7 sheep were returned to a basic ration of 1 kilogram lucerne hay daily without any further supplementary feeding.

During all three periods samples of ruminal material were repeatedly withdrawn from all the animals during the early morning and test fermentations of glucose conducted *in vitro* as described.

In Table 8 data are presented indicating the average gas production from samples of rumen material collected from the individual sheep during the second half of each of the three periods, i.e., after the animals had become fully accustomed to the particular diet.

TABLE 8.

Influence of Diet on Fermentative Activity of Ruminal Fluid.

Period.	Sheep No.	Diet.	Average Amount of Gas Prodused (ml.), with 1 ml., 20 Per cent. Glucose (30 Minute Period).
			Ml.
1	1	Lucerne and sugar	29
	2	Lucerne and sugar	36
	3	Lucerne and maize	26
	4	Lucerne and maize	35
	5	Lucerne	30
	6	Lucerne	28
	7	Lucerne	31
2	1	Teff and sugar	14
	2	Teff and sugar	17
	3	Teff and maize	10
	4	Teff and maize	12
	5	Teff	6
	6	Teff	6
	7	Teff	9
3	1	Lucerne only	22
	2	Lucerne only	27
	3	Lucerne only	29
	4	Lucerne only	26
	5	Lucerne only	29
	6	Lucerne only	26
	7	Lucerne only	27

These findings indicate that the supplementary feeding either of cane sugar or of starch in the form of crushed maize produces little if any change in the activity of the ruminal fluid while animals are kept on a basic ration of lucerne hay. When, however, teff hay is substituted in place of lucerne, there follows a decided alteration of the ruminal ingesta in that its power to ferment glucose is severely depressed. This is especially marked in two out of three control animals (Nos. 5-7) in which gas production becomes very low.

V. The Nature of the Enzyme System in Ruminal Ingesta Responsible for Fermentation of Sugars.

Following the demonstration of the rapidity with which glucose, fructose and sucrose are fermented by ruminal fluid as expressed in the volume of gas liberated, an attempt was made to locate and identify the factor responsible for this process. For this purpose freshly drawn and strained ruminal fluid was used. Microscopically this revealed the presence of a wide variety of micro-organisms including several species of infusoria, many species of bacteria, pseudo-yeast cells, and mould spores. An account of some of these organisms and their respective activities is presented in publications by Baker and Martin, and more recently by van der Wath. On a diet consisting exclusively of lucerne hay this fluid assumed a densely turbid yellowish green colour. On standing, a finely flocculent green precipitate settled out within 30 minutes leaving a less turbid yellowish grey supernatant fluid. The precipitate was found to be comprised largely of chlorophyll which formed a thick colloidal mass.

Tests carried out on this supernatant fluid showed that the fermentation of glucose was as active as that in the original material, thereby indicating that the enzyme system was not adsorbed by the colloidal chlorophyll which could be discarded after its precipitation. Most of the infusoria, moreover, settled out with the chlorophyll and frequently formed a distinct, milky-white layer at the bottom of the flask. The remaining infusoria, mostly of the smaller varieties, could be readily thrown down by slow speed centrifugation (± 500 r.p.m.) within one minute. As the supernatant fluid still retained full activity, this afforded evidence that the infusoria were not associated with this process of rapid sugar fermentation. By centrifugation of supernatant fluid for variable periods further aliquot samples of fluid were obtained for the fermentation tests. In this case centrifugation was carried out at a speed of 3,000 r.p.m. which resulted in the progressive precipitation of a thick, greyish, white slimy mass and a decrease in the turbidity of the supernatant fluid.

Table 9.

Gas Production (ml.) from Supernatant Ruminal Fluid (50 ml.) Centrifuged for Variable Periods. 1 ml. 20 per cent. Glucose subsequently added.

Duration of Fermentation.	Duration of Centrifugation (3,000 r.p.m.).				
	Normal Material.	1 Minute.	2½ Minutes.	5 Minutes.	30 Minutes.
10 minutes................	15·5	7·8	5·6	4·8	3·0
20 minutes................	9·0	3·2	1·9	1·5	1·4
30 minutes................	3·0	0·9	0·7	0·5	0·9
Total gas in 30 minutes....	27·5	11·9	8·2	6·8	5·3

These results indicate that at a speed of 3,000 r.p.m. the activity of the supernatant fluid is promptly reduced from a value of 27·5 ml gas to 11·9 ml. within one minute of centrifugation. This is followed by a slowly progressive decrease to 5·3 ml. after 30 minutes. From the ease with which the main part of the active agent could be precipitated, it was concluded that the rapid fermentation of glucose thusfar recorded both in the rumen and *in vitro* tests, was associated not with a free enzyme system but with cellular elements of relatively large size. This was confirmed by microscopic examination of the precipitate which was found to be comprised of a dense mass of clear oval-shaped cellular organisms with an average size of $8 \times 4\mu$. On the other hand the number of bacteria, comprised mostly of small rods and cocci, showed no significantly greater concentration in the precipitate than in the supernatant fluid, due evidently to insufficient centrifugation. By suspending these oval shaped cells, after being washed in water three times, in a solution of 0·2 per cent. sodium bicarbonate, it was found that the addition of glucose was followed by rapid gas production similar to that noted with the untreated ruminal fluid. Thus it could be shown that the precipitate from 50 ml. ruminal fluid when suspended in 50 c.c. 0·2 per cent. $NaHCO_3$ to which 1 ml. of 20 per cent. glucose was added produced 25 ml. of gas with 30 minutes.

The staining of small quantities of the material with equal amounts of Gram's iodine solution, both before and after the addition of glucose, resulted in a rapidly developing brown discoloration which became noticeable within 5 minutes following the addition of glucose. When stained with iodine solution after standing in the water bath for 30 minutes at 39° C., the material changed to a dark chocolate brown colour with a tendency towards rapid sedimentation of the darkened particles. Microscopically this proved to be the oval cells described above. They appeared to be distended with a homogeneous dark brown material when stained after the addition of glucose. In contrast they were of a clear hyaline appearance in the absence of sugar. From this staining reaction and also from subsequent chemical analysis it was evident that these cells were capable of rapidly transforming glucose into glycogen which was stored for variable periods within their cytoplasm. Moreover, this synthesis of glycogen was associated with a fulminating type of gas production, the peak period of which was reached within ten minutes after the addition of glucose. This was followed by a distinct "break" in the gas curve, marked by the onset of a second phase, during which there was a prolonged evolution of minute amounts of gas only. Similar organisms were found in the ruminal ingesta of all sheep on an adequate diet, although there was evidence of wide variation in their numbers in the different animals. Likewise the type of diet was found to exert an important influence, as larger concentrations of these organisms were noted with lucerne feeding than on a ration comprised of poor quality grass-hay, the feeding of which resulted in a rapid dwindling down of their numbers.

From its structure and general behaviour this organism was clearly related to the yeasts. It could, however, be differentiated from true yeasts (Saccharomyces species) in that multiplication took place by binary fission after the appearance of a faint cleavage line across the centre. This was followed by the liberation of two identically shaped daughter cells instead of the typical bud formation as seen in true yeasts. In regard to its morphological characteristics it showed a very close resemblance to the false yeast, *Schizosaccharomyces octosporus* as described by Beyerinck. Accordingly this

species encountered in the rumen ingesta of sheep was named *Schizosaccharomyces ovis*. (See Figure 1.) Similarly shaped yeast cells although of larger size were found in the ruminal ingesta of cattle. Whether this constitutes a distinct species from that seen in sheep remains to be decided.

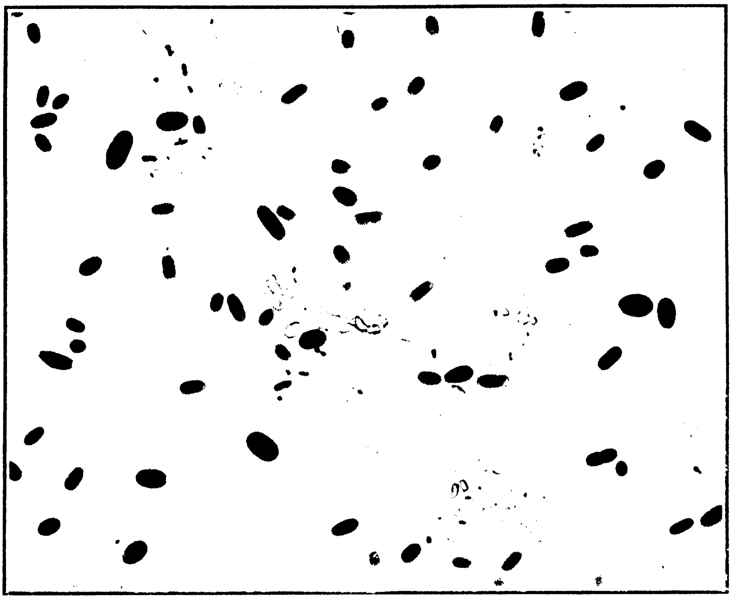


FIG. 1. Pseudo yeast cells (Schizosaccharomyces ovis) from rumen of sheep. Stained with Grams iodine soln. to show glycogen content of cells. Enlargement 640×.

A. *Influence of Starvation on Yeast Cells in Ruminal Ingesta.*

In this experiment five sheep with rumen fistulae were kept on a daily diet comprised of 1 kilogram dried lucerne hay per sheep. Repeated fermentation of glucose added to fresh samples of rumen ingesta revealed the characteristic fulminating type of gas development which after reaching its peak within the first ten minutes, rapidly subsided in the following twenty minutes. In every instance this was associated with a definite synthesis of glycogen in the yeast cells, large numbers of which were present in the ingesta of all five sheep. Added to the formation of glycogen by the yeast cells, there was evidence of an equally rapid synthesis of polysaccharide (probably starch) from glucose by various species of iodophilic cocci and small rods resembling those described by Baker and Martin. This was revealed by the intense blue staining of these organisms with iodine solution in contrast to the dark brown colour assumed by the yeast cells. The extent to which starch was synthesised, judging by the starch-iodine reaction, was, however, completely overshadowed by the numerous yeast cells, heavily laden with glycogen.

Following the above observations, all animals were completely starved for a period of three days after which they were returned to their normal diet of lucerne. Meanwhile, fermentations and microscopic examinations

were carried out daily on ingesta collected from each animal. After starvation for 24 hours there was a decided decrease in the gas yield from all the ruminal samples which coincided with a drop in the number of yeast cells and the amount of glycogen formed. On the second, and even more so, on the third day of starvation, only minute traces of gas were produced from the rumen material while the number of yeast cells and with it the amount of glycogen formed also showed a very sharp decrease. Following the return to their lucerne diet, 4 out of 5 animals consumed only half their daily ration of 1 kilogram on the first day. By this time yeast cells had completely disappeared from the rumen ingesta of all sheep. On the fourth day all animals were still consuming less than their usual ration. There was, however, evidence of increased gas production from glucose although yeast cells, and with them glycogen formation, still remained absent. Instead there appeared vast numbers of iodophilic cocci which showed a rapid synthesis of starch from the glucose added. Moreover, the gas curve, instead of the usual fulminating type, lacked its high peak period during the first ten minutes. In this it was replaced by a slow rate of gas evolution which was maintained at a practically even level after the 30 minute test period. It was on the sixth day only, following the end of starvation, that all animals consumed their full ration. At the same time a few yeast cells were just reappearing in the ingesta from two sheep. Gas production remained slow although at a slightly higher level than on the previous days. This was associated with intense starch synthesis from the test glucose by the greatly increased numbers of iodophilic cocci which appeared to be firmly established in the rumen. In 4 out of the 5 sheep yeast cells apeared fully re-established in the ruminal ingesta only after a period of 14 days. This was accompanied by the return of extensive glycogen synthesis from glucose and a more rapid rate of gas production. On the other hand the synthesis of starch from sugar by the iodophilic micro-organisms was effectively suppressed in the presence of increasing numbers of yeast cells. These observations afforded very striking evidence of the keen competition displayed by the different ruminal organisms for sugar and also of the manner in which it was utilized by the various groups. Moreover, they demonstrated that the fermentation of sugars by ruminal organisms is associated both with the production of variable amounts of gas as well as with the synthesis of new carbohydrate which is stored within their cytoplasm. In sheep on a diet of lucerne hay the most prominent organism in this respect is the species of false yeast described above. Through its presence, part of the glucose added to fresh ruminal ingesta, when shaken at 39° C. in the presence of air, undergoes extremely rapid oxidation with the production of large volumes of gas. Simultaneously with this, another fraction of the glucose is actively assimilated by the yeast cells and synthesised into glycogen which is stored as reserve carbohydrate. Following suppression of this yeast strain through starvation of the host animal, the yeast cells are readily supplanted by a dense culture of strongly iodophilic cocci as soon as feeding is resumed. These organisms are responsible for an active synthesis of starch from glucose rather than that of glycogen as noted in the yeast cells. Gas production also is at a lower level although maintained for relatively longer periods. With the subsequent re-establishment of the yeast population in the rumen there follows a regression in the synthesis of starch from sugar by the iodophilic microflora.

Concerning the significance of this carbohydrate synthesis by ruminal organisms either as a source of energy or in the synthesis of vitamin B and other compounds, further research work is to be undertaken.

B. *Carbohydrate Assimilation in Other Organisms.*

From investigations on the colourless alga, *Prototheca Zopfi,* Barker was able to demonstrate a rapidly occurring assimilation of such organic compounds as glycerol, ethyl alcohol, glucose, acetic-, propionic-, butyric-acids and valeric acid by cell suspensions of the alga. This process of oxidative assimilation as it was termed, coincided with the consumption of oxygen and the production of carbon dioxide in amounts varying with the nature of the substrate. During the primary phase of the reaction the substrate was rapidly converted through oxidation into a carbohydrate and stored as glycogen within the cells. This was followed by a slowly proceeding secondary phase during which glycogen breakdown became associated in the processes of cell synthesis. Depending on the substrate, from 50 to 80 per cent. of its carbon was assimilated in this manner.

Results essentially similar to those reported by Barker were obtained by Clifton and Logan in studies on washed suspensions of *Escherichia coli.* Thus oxidation of such substrates as acetate, lactate, propionate, glycerol and glucose instead of being carried to completion resulted in the assimilation of a portion of the substrate as carbohydrate by the cells. Moreover, there was evidence that the processes of dissimilation (respiration) and assimilation were closely connected and probably of the nature of coupled reactions. As the amount of oxygen consumed during the primary phase of the reaction always forms a constant proportion of the total amount required for oxidation of a particular substrate it was concluded that the ratio of synthesis to oxidation remained constant for any given system. In the presence of suitable concentrations of sodium azide (NaN_3) or of alpha dinitrophenol, assimilation was inhibited while the oxidation of the substrate was allowed to proceed to completion. A similar type of poisoning was caused by monoiodoacetate which according to the findings of Brücke inhibited the formation of glycogen by yeast both from glucose and ethyl alcohol. Likewise, Clifton found that oxidative assimilation by *Pseudomonas Calco-acetica* and *Escherichia coli* was prevented by iodoacetate.

According to the findings of Winzler and Baumberger a large percentage of glucose which disappeared from a yeast suspension in the presence of oxygen was synthesised to intracellular carbohydrate. In this manner three-fourths of the glucose was stored while the remaining one-quarter was oxidised. Likewise 58 per cent. sodium acetate was oxidised while the remaining 41 per cent. was synthesised. In the absence of air as in alcoholic fermentation on the other hand, 70 per cent. was fermented while only 30 per cent. became stored by the yeast cells. There is, however, as yet no unanimity concerning the various phases of sugar fermentation. Thus Willstätter and Rohdewald claim that the synthesis of polysaccharides, mainly as glycogen, forms an essential stage in the fermentation of glucose and maltose prior to the process of phosphorylation. Kruyk and Klingsmuller, on the other hand, noted a rapid disappearance of glucose from yeast suspensions without corresponding synthesis of any glycogen. According to the findings of Goda, addition of sugar to fresh beer yeast results in a brief induction period during which glycogen accumulates within the cells. In older cultures, however, no such glycogen formation could be demonstrated. In experiments on pure cultures of *Saccharomyces cerevisiae*, van Niel and Anderson found that as much as 30 per cent. of added glucose was initially converted into complex carbohydrates, while the production of carbon dioxide and ethyl alcohol accounted for the remaining 70 per cent. This was interpreted as further evidence of the occurrence of fermentative assimilation.

However, this could not be demonstrated in lactic acid fermentation. Concerning the nature of the carbohydrates synthesised by yeast, it has been definitely established that apart from glycogen, other compounds such as yeast gum and membrane polyose are also formed. According to the work of McAnally and Smedley-Maclean the addition of phosphate to glucose or maltose media increased the storage of glycogen, yeast gum and insoluble carbohydrate by yeast cells. The amount of glycogen formed from maltose far exceeded that derived from glucose.

The foregoing literature, therefore, affords strong evidence that carbohydrate metabolism as studied in a variety of micro-organisms including yeast strains, is closely associated with the phenomena of oxidative or fermentative assimilation. By these processes variable amounts of added carbohydrate are either assimilated by the organisms and synthesised into reserve carbohydrate (glycogen) or rapidly oxidised with the production of gas (CO_2) and water. The relative extent to which either assimilation or oxidation proceeds, as well as the speed of reaction in these two phases of bacterial metabolism, depends on a variety of factors in which the type of organisms, the amount and nature of their food supply, and the degree of aeration appear to be of the greatest significance. The present investigations of the yeast strain *Schizosaccharomyces ovis* as well as other species of iodophilic micro-organisms, have revealed the fact that fundamentally similar processes of oxidative assimilation are of normal occurrence within the forestomachs of sheep and probably also in other species of ruminants.

CONCLUSION.

In studies conducted on merino sheep with permanent ruminal fistulae, it has been demonstrated that acute gas production in the forestomachs immediately after the consumption of certain foods is associated with a process of oxidative assimilation. By this process variable proportions of such sugars as glucose, fructose, and sucrose are rapidly oxidised through the agency of a strain of false yeast, *Schizosaccharomyces ovis*, which is present in large numbers in the rumen of sheep, especially when such animals are kept on a diet of lucerne. Attending this oxidation of part of the ingested sugar, large volumes of gas are suddenly generated within the ruminal mass. Simultaneously with this, the rest of the sugar is rapidly assimilated and stored as glycogen by the yeast cells. Complete starvation or inadequate feeding of the animal is promptly followed by suppression leading up to a total disappearance of this yeast strain. Under these circumstances various iodophilic bacteria normally present in the ruminal ingesta are afforded the opportunity of metabolising the available sugar. This is associated with the synthesis of starch by these organisms instead of glycogen. Moreover, oxidation shows greater restriction as is evident from the reduced amount of gas produced.

While this extensive synthesis of glycogen and other polysaccharides forms an integral part in the carbohydrate metabolism of various ruminal micro-organisms, its full significance in the biology of the microflora and especially in the nutrition of the host animal itself is as yet not fully understood. In view of the close relationship existing between ruminant digestion and bacterial activity there are indications, however, that the nutrition of ruminant animals is vitally linked with various products derived from bacterial metabolism, hence the necessity of further investigations in this field.

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Studies on the Alimentary Tract of Merino Sheep in South Africa VIII.—The Pathogenesis of Acute Tympanites (Bloat).

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In spite of considerable attention devoted to the problem of acute tympanites in ruminants, the aetiology and the pathogenesis of this condition is as yet poorly understood. One of the most commonly accepted views is that it is due to the formation of a food plug in the distal part of the oesophagus, thereby obstructing the normal eructation of gas from the forestomachs. McIntosh in a recent publication refers to two types of acute bloat—

(*a*) in which free gas is superimposed on the ruminal mass, its escape being impeded by obstruction in the oesophagus, and

(*b*) where the gas remains admixed in the ruminal ingesta as noted after the engorgement with succulent food.

In regard to the actual causes of acute bloat McIntosh admits that these are unknown, but states that chemical factors within the plant are important in view of the experience that the condition is less frequently encountered on lucerne pastures in which fertilization and irrigation have been well maintained, than is the case on neglected pastures growing on poor soil. From analyses conducted on ruminal gas, Dougherty on the other hand concludes that bloat is associated with the appearance of distinct amounts of carbon monoxide and sulphuretted hydrogen which may be the cause of paralysis of the rumen, and thus to its overdistension with gas.

Due to its insidious nature, it is extremely difficult to predict the onset of acute bloat even though it is known that it is usually associated with the feeding of lucerne, or less commonly with other legumes. Moreover, no specific substance has as yet been obtained from lucerne which could be incriminated as the cause of the condition. That this factor is of a very labile character is indicated by the suddenness with which the green plant may become dangerous to ruminants, whereas hay made from it is usually quite harmless. On the other hand, the animal itself may constitute a determining factor as shown by the fact that certain individuals are far more subject to bloat than are others feeding on the same material. Likewise, ravenous feeding, as noted in hungry animals, frequently precipitates the condition.

Experimental Production of Bloat.

In order to study the pathogenesis of the symptom complex in acute bloat, a series of experiments were undertaken. In the first of these, both cattle and sheep were placed on a diet consisting largely, or in other cases

exclusively, of green lucerne in various stages of growth, the plants being either grazed down or cut and subsequently fed from troughs. Moreover, attention was also paid to the degree of wilting of the lucerne at the time that it was being fed.

The results of the various trials indicated that although lucerne feeding usually led to a rapid filling out of the abdomen, the degree of distension displayed was not accompanied by the characteristic signs of dyspnoea and general distress as noted in cases of acute bloat. Nevertheless, there was definite evidence of an increase in intra-ruminal pressure immediately after a meal of lucerne. This was shown by the forced ejection of ingesta through the ruminal fistula on being opened during this period. No such forced ejection was, however, displayed some hours after the consumption of the lucerne nor when animals were kept on a diet of grass hay supplemented with ground maize.

To ascertain whether the rate of gas production within the rumen could in itself determine the onset of acute bloat, the forestomachs of several sheep were insufflated with air. This was carried out by connecting the ruminal fistula tube to a large pressure cylinder from which a regulated stream of air could be passed into the rumen. In every instance it was found that such animals were capable of belching as much as six litres of air insufflated per minute. Additional raising either of the forequarters or of the hindquarters or even flooding of the rumen with an extra three litres of water, made very little difference to the ease with which animals were able to expel the large volumes of air constantly rushed into the rumen. These experiments afforded clear evidence that the rate of gas production in the rumen could not in itself determine the onset of bloating. Similarly they served to demonstrate the remarkable mechanism whereby the ruminant animal was safeguarded against overdistension of its forestomachs under normal conditions.

Foam Formation in the Rumen.

Repeated examinations made on fresh ruminal ingesta which was drawn from animals kept on different diets, revealed a definite tendency towards foam formation in those fed on lucerne, whereas in sheep on other diets this was at most only slight and of a transitory nature. Moreover, the foam present in the lucerne ingesta when kept standing at room temperature, required a period of several hours before the gas bubbles collapsed. Similar effects were observed both in pressed lucerne juice and watery extracts made from lucerne hay, which on being vigorously shaken, showed a copious formation of relatively stable foam still evident after a period of 12 hours. Comparative tests conducted on similarly prepared extracts and juice from oats and barley failed to produce this characteristic stable foam which closely resembled that seen in saponin solutions when shaken up. According to the findings of Jacobson, who undertook a series of chemical investigations on lucerne, a saponin with particularly strong foam-producing qualities could be isolated from this plant. This was confirmed in the present investigations undertaken in collaboration with de Waal. As described by Jacobson a characteristic non-haemolytic saponin, but with very strong foam-producing qualities could be isolated from all samples of lucerne analysed. To ascertain whether, in addition to the saponin in lucerne, the presence of certain colloids would aggravate foaming, sugar fermentation tests were conducted on ingesta to which varying amounts

either of egg albumen or gum acacia had been added. There was, however, no definite evidence of a change in foam production following the addition of these colloids.

Observations made on Cases of Clinical Bloat.

As a result of the difficulties experienced in reproducing cases of clinical bloat under laboratory conditions, the investigations were extended to the Losperfontein Experimental Station where trials were in progress in regard to fat lamb production on different pastures. On various occasions outbreaks of clinical bloat had made their appearance during the period that animals were restricted to lucerne pasture. It was during one of these outbreaks that, in collaboration with Starke, detailed observations were made on the condition. The following represents the main points established during this outbreak:—

1. Cases of rapidly fatal bloat could be definitely caused by the ingestion of fresh green lucerne in an unwilted, unbruised condition and in the preflowering stage.

2. It was associated with a ravenous consumption especially of the green leafy tops, in contrast to the more fibrous stems which were left standing.

3. It was definitely more evident amongst lactating ewes showing a keen appetite than amongst others feeding less greedily.

4. According to analyses made by Louw, the sugar content of the lucerne collected during the actual outbreak increased from 2·5 per cent. in the early morning to 6 per cent. (on dry weight basis) in the late afternoon. Cases of fatal bloat were definitely more numerous in the late afternoon even though actual grazing on lucerne had been restricted to a period of half an hour only.

5. Abdominal distension especially evident in the left flank, was such as to cause complete immobilization of the diaphragm with its dome extending far forward into the thoracic cavity. The resultant asphyxia, combined with obstruction in the return of venous blood from the posterior vena cava, was responsible for the acute death.

6. Opening of the abdominal cavity and of the rumen at the moment of death failed to reveal constriction in the distal part of the oesophagus.

7. The ingesta which was held under great pressure within the rumen consisted of a strongly foaming, frothy mass. Moreover, there was little tendency for free gas to escape from this rapidly fermenting material thus explaining the limited value of ruminal puncture in such cases. Lucerne leaves which comprised the bulk of the solid material showed definite signs of fermentative disintegration.

8. Microscopically, the ingesta from every rumen examined revealed the presence of a dense culture of the pesudo-yeast strain described in the previous report. (Quin, 1943.)

Oxidative Assimilation in the Forestomachs in relation to Acute Bloat.

From the above observations, when considered in conjunction with the investigations previously outlined, the following explanation concerning the aetiology and pathogenesis of acute bloat in ruminants appears to be justified.

Clinically, acute bloat represents an integral part of the normal processes associated with sugar metabolism in the forestomachs of ruminants. Due to the activity of yeast cells (*Schizosaccharomyces species*) in the rumen, a dense culture of which is established on a diet of lucerne, a portion of the ingested plant sugar is immediately assimilated and stored by these cells, mainly as glycogen; the remaining sugar is exposed to a fulminating oxidation and the production of large volumes of gas, comprised chiefly of carbon dioxide. This process, referred to as oxidative assimilation, has been established in the biology of various unrelated micro-organisms and represents a characteristic feature in their carbohydrate metabolism. In acute tympanites, the balance normally established between the rate of gas production in the forestomachs and its escape through the oesophagus may be suddenly disturbed, frequently with fatal consequences. The condition arises through a combination of (*a*) the presence of characteristic pseudo-yeast cells in the rumen, (*b*) the rapid consumption of fresh lucerne leaves showing an elevated sugar content, (*c*) the establishment of an aerobic state in the rumen associated with rapid swallowing of food thus promoting excessive oxidation of sugar and gas formation, (*d*) the presence of saponin in lucerne which by increasing surface tension and the tendency towards foam formation, impedes the escape of gas from the ruminal mass.

The eructation of foam from the rumen presents serious difficulties, in contract to the easy escape of free gas. Consequently the excessive frothiness created within the ruminal ingesta leads to unphysiological distension in the forestomachs through which both respiration and circulation are endangered.

Control of Acute Bloat.

In view of the findings that the pathogenesis of acute bloat is intimately associated with various complicated physiological phenomena in the forestomachs of ruminants, the successful control of this condition depends more upon a rational feeding practice rather than on the administration of remedies calculated to suppress fermentation in the rumen. In a subsequent report consideration will be given to the susceptibility of the ruminal flora to a variety of poisons and other foreign substances.

Due to the fact that starvation readily leads to a depletion of the glycogen reserves within the yeast cells of the rumen, it is essential to safeguard ruminant animals against spells of acute hunger, especially where green succulent lucerne constitutes the major item in their normal diet. Under such conditions the ingestion of green lucerne is immediately associated with an accentuated phase in the assimilation and oxidation of the plant sugars by the starving yeast cells, thus establishing the tendency towards acute bloat. Alternatively, the regular supplementation of carbohydrate rich foods such as grain, molasses or good quality hay, with the lucerne diet, keeps the energy demands of the yeast cells more or less satisfied. Consequently the sugars present in lucerne are not metabolized at the same dangerously high speed, with the result that gas and foam production are kept within physiological bounds.

The same result is achieved in the majority of animals where undisturbed fulltime grazing is allowed on lucerne pastures. Through the cultivation of desirable feeding habits whereby small quantities of lucerne are repeatedly consumed rather than excessive amounts during a single meal, conditions within the forestomachs remain fairly well stabilized, and hence

the dangers of acute bloat are minimized. In this connection attention should be directed to the increased degree of aeration of the ruminal ingesta especially during the hurried consumption of succulent leafy material such as green lucerne. Seeing that the microflora and fauna in the forestomachs are largely aerobic in character, the nature and speed of the metabolic processes occurring within these compartments are influenced by the extent of aeration and thus also by the carbon dioxide tension of the ruminal mass. Likewise, a portion of the stomach contents is periodically aerated in the open mouth during the process of rumination.

Apart from the rôle played by lucerne in the causation of acute bloat, the feeding of ruminants exclusively on fresh green lucerne may be associated with other undesirable consequences. Thus the rapid filling of the rumen with a foaming watery mass, even after the consumption of relatively small amounts of lucerne, would tend to limit the daily intake of total solids to amounts inadequate for normal body requirements. This is a matter of significance especially in the feeding of dairy cows and also of sheep concerned in fat lamb production. Repeated filling of the forestomachs with a foaming mass instead of with solid nutriment, forcibly suppresses appetite until deflation of the rumen has ensued. Alternatively, it may endanger the life of those animals, which, through ravenous feeding, fail to appreciate the first warning of undue distension through foam formation in the forestomachs.

Summary.

Concerning the pathogenesis of acute bloat, this has been shown to be closely associated with the production of gas during the rapid oxidation of sugar mainly by yeast cells as described above. Normal eructation of gas may be impeded as a result of excessive foam production in the forestomachs especially when animals are restricted to a diet of green lucerne. This foaming up of the ruminal mass is directly attributable to the presence of saponin in lucerne which through its action on surface tension retards the breaking of the foam. Undue frothing of the ruminal ingesta can be controlled by resorting to a feeding practice in which green lucerne is supplied to cattle and sheep only after the consumption of other foods.

In a subsequent report attention will be devoted to hydrogen-ion concentration in relation to fermentation in the forestomachs.

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Studies on the Alimentary Tract of Merino Sheep in South Africa IX.—The H-ion Concentration in the Forestomachs of Fistula Sheep under Different Experimental Conditions.

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INTRODUCTION.

CONSIDERABLE attention has been paid by various workers to the pH in the forestomachs of ruminants. Most of the determinations were made on material collected after slaughtering. Thus Schwarz and Gabriel (1924) found the ruminal ingesta in cattle constantly alkaline with an average value of pH 8·89. In contrast, Knoth (1928) using a different sampling technique whereby the necessary precautions were taken to exclude aeration, found the ruminal ingesta weakly acid, with an average pH of 6·9. He stressed the point that, on stirring the samples to allow aeration and liberation of CO_2 to take place, alkaline values were obtained for the same material with an average pH of 7·9. Other workers, employing the stomach tube to aspirate ingesta from the rumen of sheep, found it to be alkaline. Ferber (1928) using a similar technique, reported an average pH value of 7·9, with a range of pH 7·6-8·5.

Recently, Monroe *et al* (1939) determined the pH of bovine rumen ingesta, where various rations were fed. On a diet of hay plus grain this was found to average pH 7·01, while on hay, grain plus silage it was pH 6·95, maize plus grain pH 6·87, blue grass pasture pH 6·47, and on lucerne pH 6·66. These determinations were made by the use of the Quinhydrone Electrode method. Olsen (1941) in a study on slaughtered bovines records an average pH value of 6·86. Recent work indicates that the reaction of the forestomachs in most cases is weakly acid, and that the type of feed consumed determines a definite pH range as shown by Monroe *et al.*

Samples obtained under abattoir conditions are far from satisfactory however, in comparison to those from fistula animals under experimental conditions in which both the composition of the diet and the methods of feeding are well known. Moreover, sampling in such cases is easy, and can be repeated whenever necessary, both before and after feeding.

Aspiration of ingesta by stomach tube on the other hand, distresses the animals, besides leading to excessive salivation and hence to undue dilution and contamination of the ruminal samples with a viscid saliva. In this report data are presented on the hydrogen-ion concentration of fresh ingesta aspirated through permanent fistulae from the rumen of Merino sheep. The animals were kept on a variety of controlled diets which in several instances included the administration of acid directly into the rumen. A further series of tests on ruminal material was carried out "*in vitro*" with the object primarily of studying the adequacy of its buffer system as well as the influence of carbohydrate fermentation on pH.

Sampling of the Ingesta.

Since the method of sampling may affect the results, it will be necessary to give a brief description of the technique which was followed. By inserting a glass tube, fitted at one end with a short length of rubber tubing, into the fistula of the sheep, small quantities of rumen ingesta could be obtained through gentle aspiration by mouth at the rubber end. Immediately afterwards small glass cylinders (10 c.c. capacity) were completely filled and suitably stoppered so as to prevent undue exchange of gas.

Measurements of pH were regularly carried out within a few minutes of sampling. Unless otherwise stated, all samples were taken in the early morning before feeding.

In order to ascertain the influence of exposure to air on the pH of rumen ingesta, some of the samples were covered with a thin layer of liquid paraffin immediately after collection, to compare with our routine method, as well as with samples of the ingesta left uncovered in open containers. The samples were taken after the animals had been fed and during a period of active digestion.

Subsequent determinations of pH yielded the following results:—

Sheep No.	Routine Method.	Covered with Liquid Paraffin.	Routine Method.	Exposed 10 mins.	Exposed 20 mins.	Exposed 30 mins.
32............	6·28	6·28	6·25	6·28	6·50	6·58
43............	5·90	5·95	6·68	6·88	7·10	7·15
58............	5·92	6·00	5·62	5·68	5·90	5·98
59............	6·10	6·15	6·60	6·92	6·98	7·02
63............	6·29	6·22	6·41	6·52	6·68	6·68
66............	6·58	6·50	5·98	5·98	6·12	6·28

Measurements of the pH of Ruminal Ingesta.

Some of the values were obtained by measuring the samples potentiometrically by the use of the saturated KCl-Calomel halfcell-Quinhydrone method and calculating the results as indicated in tables 1-7. Where Quinhydrone was added to the samples, the necessary precautions were taken in recording the readings. According to Yoshimura (1936), readings may be considered accurate when recorded within 1-5 minutes on aqueous buffered solutions in the vicinity of pH 8·0. The Quinhydrone electrode applied to alkaline buffers, amino-acids, proteins and plasma causes a drift, with the

result that readings are inaccurate after a 5 minute period. On some samples (Tables 8 and 9), the pH was measured by means of the Beckman Glass-electrode apparatus, which was found to yield very accurate data when compared with the results obtained by the use of the hydrogen-electrode.

EXPERIMENTAL RESULTS OBTAINED WITH FRESH RUMINAL INGESTA.

In order to study the influence of diet on the pH of the ruminal ingesta, a total of 23 fistula sheep were used at various times while being kept on one of the following daily diets:—

(*a*) Lucerne Hay ... ... 300 grams.
Crushed Maize ... ... 360 grams.
(*b*) Lucerne Hay ... ... 1 kilogram.
(*c*) Crushed Maize ... ... 500 grams.
Lucerne Hay ... ... 100 grams.
(*d*) Green Lucerne (fresh) ... ... 1 kilogram.
(*e*) Crushed Maize ... ... 600 grams.
(*f*) Mature Grass Hay ... ... 400 grams.

TABLE 1.

pH of ruminal ingesta of fistula sheep.

Diet: Lucerne Hay (C) ... ... 300 grams daily
Crushed Maize ... ... 360 grams daily

Sheep No.	Number of Samples.	pH Variations.	Average pH Variation for 5 Sheep.
36	6	6·4–7·1 (av. 6·8)	6·4–7·1
37	7	6·4–6·8 (av. 6·6)	
43	4	6·7–7·0 (av. 6·9)	
49	4	6·8–6·8 (av. 6·8)	
50	4	6·6–6·8 (av. 6·7)	

TABLE 2.

pH of ruminal ingesta of fistula sheep.

Diet: Fresh Green Lucerne ... ... 1 kilogram daily.

Sheep No.	Number of Samples.	pH Variations.	Average pH Variation for 2 Sheep.
32	7	7·4–7·6 (av. 7·5)	7·3–7·7
35	5	7·3–7·7 (av. 7·5)	

TABLE 3.

pH of ruminal ingesta of fistula sheep.

Diet: Crushed Maize ... ... 600 grams daily.

Sheep No.	Number of Samples.	pH Variations.	Average pH Variation for 2 Sheep.
37..............................	8	5·3–6·2 (av. 5·8)	5·3–6·2
45..............................	8	5·4–5·9 (av. 5·7)	

TABLE 4.

pH of ruminal ingesta of fistula sheep.

Diet: Crushed Maize ... ... 500 grams daily.
Lucerne Hay (B) ... ... 100 grams daily.

Sheep No.	Number of Samples.	pH Variations.	Average pH Variation for 2 Sheep.
14..............................	11	5·6–6·5 (av. 6·1)	5·5–6·8
17..............................	11	5·5–6·8 (av. 6·2)	

TABLE 5.

pH of ruminal ingesta of fistula sheep.

Diet: Mature Grass Hay (poor quality) ... ... 400 grams daily.

Sheep No.	Number of Samples.	pH Variations.	Average pH Variation for 8 Sheep.
20..............................	6	6·8–7·2 (av. 7·2)	6·8–7·6
21..............................	6	6·9–7·5 (av. 7·2)	
23..............................	6	6·8–7·3 (av. 7·1)	
25..............................	6	6·8–7·6 (av. 7·2)	
25..............................	6	6·8–7·6 (av. 7·2)	
26..............................	6	6·8–7·6 (av. 7·2)	
27..............................	6	6·8–7·6 (av. 7·2)	
28..............................	6	6·9–7·5 (av. 7·2)	
29..............................	6	6·8–7·6 (av. 7·2)	

TABLE 6.

pH of ruminal ingesta of fistula sheep.

Diet: Changed from Mature Grass Hay to
Lucerne Hay (B) ... ... 1 kilogram daily.

Sheep No.	Number of Samples.	pH Variations.	Average pH Variation for 2 Sheep.
28..............................	12	5·8–7·5 (av. 6·7)	5·8–7·5

Table 7.

pH of ruminal ingesta of fistula sheep.

Diet: Changed from Mature Grass Hay to:—
Lucerne Hay (B) ... ... 300 grams daily.
Crushed Maize ... ... 360 grams daily.

Sheep No.	Number of Samples.	pH Variations.	Average pH Variation for 6 Sheep.
20	14	5·8–7·1 (av. 6·5)	5·8–7·3
21	14	6·2–7·1 (av. 6·7)	
23	14	5·8–7·1 (av. 6·5)	
25	14	5·8–7·0 (av. 6·4)	
26	14	6·2–7·3 (av. 6·8)	
29	14	6·1–7·3 (av. 6·7)	

Table 8.

pH of ruminal ingesta of fistula sheep, sampled at various periods of digestion.

Diet: Lucerne Hay (C) ... ... 300 grams daily.
Crushed Maize ... ... 360 grams daily.

B.F. = Before feeding. A.F. = After feeding. Hr. = Hour.

Sheep No.	Average pH Variations on Consecutive Days.								
	B.F.	$\frac{1}{4}$ Hr. A.F.	$\frac{1}{2}$ Hr. A.F.	1 Hr. A.F.	$1\frac{1}{2}$ Hrs. A.F.	$2\frac{1}{4}$ Hrs. A.F.	$4\frac{3}{4}$ Hrs. A.F.	6 Hrs. A.F.	24 Hrs. A.F.
43	6·12–6·50	—	—	6·00–6·60	—	—	—	6·42–6·90	6·12–6·50
59	6·32–7·05	—	—	6·50–7·18	—	—	—	6·20–7·40	6·32–7·05
43 (4 days)	6·15–6·65	5·85–6·12	5·70–6·08	6·00–6·15	6·00–6·22	5·75–6·02	6·12–6·12	5·80–6·40	6·20–6·72
59 (3 days)	6·00–6·72	5·80–6·28	5·65–5·98	6·00–6·15	5·95–6·02	5·96–6·00	6·05–6·60	6·52–6·62	6·10–6·72

Table 9.

pH of ruminal ingesta of fistula sheep, sampled at various periods of digestion.

Diet: Lucerne Hay (C) ... ... 1 kilogram daily.

B.F. = Before Feeding. A.F. = After Feeding. Hr. = Hour.

Sheep Nos.	Average pH Variations for 5 Sheep on Consecutive Days.						
	B.F.	$\frac{1}{4}$ Hr. A.F.	1 Hr. A.F.	$2\frac{1}{4}$ Hrs. A.F.	$4\frac{3}{4}$ Hrs. A.F.	6 Hrs. A.F.	24 Hrs. A.F.
39, 58, 59, 63, 66	6·45–6·72	—	6·12–6·32	6·11–6·36	5·90–6·36	6·21–6·45	6·63–6·95
39, 59, 59, 63, 66	6·63–6·95	6·38–6·58	6·20–6·51	6·00–6·42	6·04–6·35	6·45–6·59	—

TABLE 10.

Composition of Feeds.

Expressed as grams per cent. on absolute dry basis.

Type of Feed.	Moisture.	Protein.	Fibre.	Ether Sol. Ext.	Total Ash.	N.-free Ext.	P.	Ca.	Mg.
Lucerne Hay (A)...............	7·4	14·1	36·0	2·1	8·9	38·9	—	—	—
Lucerne Hay (B)...............	6·1	16·3	31·5	2·2	8·1	41·9	·16	1·93	·60
Lucerne Hay (C)...............	9·6	19·2	25·0	2·2	9·6	44·0	·25	1·46	·52
Green Lucerne..................	75·3	16·3	31·0	3·0	8·0	41·7	—	—	—
Yellow Maize..................	9·9	10·9	1·9	3·1	3·1	72·8	·24	·025	·12
Mature Grass Hay..............	5·4	4·6	41·6	1·5	6·1	46·1	·06	·27	·12

As will be noted from the above tables Nos. 1-7, the pH values recorded for rumen ingesta on the different diets are on the acid side in the majority of cases. Where the ration was comprised of excessive amounts of starchy material e.g. crushed maize, the pH was more definitely acid with a range of pH 5·5-6·8. In a few animals kept exclusively on crushed maize, the pH showed a further decrease to pH 5·3-6·2, the ingesta developing an excessively sour, cheesy odour. This was followed ultimately by a complete loss of appetite and other digestive disturbances.

Where this maize diet was supplemented with lucerne hay, the pH ranged from 6·4-7·1. On a ration of fresh green lucerne only, the values were slightly alkaline with a pH range of 7·3-7·7.

Exclusive feeding of dry lucerne hay, however, resulted in a slightly acid ingesta with the pH ranging from 6·45-6·95.

Well matured grass hay cut from the open veld and of low nutritive value, yielded ruminal ingesta, verging on the alkaline side with a pH range of 6·8-7·6. When the sheep which had subsisted on this poor grass hay for a considerable time, were placed on lucerne hay to improve their condition, the pH of the ingesta, after the animals had been established on the lucerne diet, was found to fluctuate between 5·8-7·5. Similarly, some of the above animals when placed on a mixed diet of lucerne hay and maize, yielded ingesta varying in pH from 5·8-7·3.

From the results presented in tables 8 and 9 it will be noted that the pH of the rumen ingesta is subjected to a very limited fluctuation during the course of digestion of a single meal, the tendency being towards greater acidity within the first 4-6 hours. After this it steadily returns to its previous level. This acidification of the ingesta is more evident after the consumption of a mixed ration containing both lucerne and maize than a meal composed of lucerne hay only.

The Buffer System of Ruminal Ingesta.

In order to maintain optimal conditions under which the normal density and the physiological activity of the various micro-organisms within the forestomachs can be safeguarded, it is essential to limit pH fluctuation as far as possible. This can be achieved only through the presence of an efficient buffer system which, as in the case of blood, is capable of ensuring relatively stable conditions despite variations in its acid and alkali content. Through constant production of organic acids mainly as a result of bacterial action on carbohydrates there is a distinct tendency towards undue acidification in the forestomachs. Normally, however, this is effectively counteracted by the free flow of an alkaline saliva containing a significant concentration of sodium bicarbonate. Together with the food proteins, the $NaHCO_3$ and phosphates of the saliva on being mixed with the food, exert the desirable buffering effect. Consequently, the type and amount of the food as indicated in the previous section normally provoke relatively small fluctuations in pH of a temporary nature.

With the object of studying the efficiency of this buffer system a series of experiments were conducted in which the pH of ruminal ingesta was correlated with its power to ferment glucose. In these tests, attempts were made to alter the pH in a variety of ways, e.g., by constant shaking, standing of ingesta at room temperature, the addition of either acid or alkali, or of carbohydrates such as molasses and glucose.

The Influence of Standing on the pH of Rumen Ingesta and on its Ability to Ferment Glucose.

After withdrawal from the same animal immediately prior to feeding, aliquot amounts of rumen ingesta of 50 c.c. each were kept standing at room temperature in open Erlenmeyer flasks. As indicated below various materials were added to these flasks. This was followed by the determination of the pH immediately afterwards and again 24 hours later.

Material Added.	Initial pH.	pH after 24 Hours Standing.
1. None	7·45	8·40
2. 0·2 gm. Glucose	7·22	7·36
3. 1·0 gm. Glucose	7·28	5·30
4. 0·5 gm. Molasses	6·96	6·91
5. 2·5 gm. Molasses	6·80	5·28
6. 5 c.c. N/10 HCl	6·66	8·16
7. 10 c.c. N/10 HCl	6·39	7·76
8. 15 c.c. N/10 HCl	6·16	7·52

After standing 24 hours at room temperature (18°-25° C), test fermentation of glucose was carried out on all the above flasks. Gas production was found to be normal only in flasks Nos. 2, 4, 7 and 8, showing a range from pH 6·91-7·76, whereas in the remaining flasks in which the H-ion concentration was either above pH 8·0 or below pH 6·0, no gas at all was produced.

From the above data it appeared that the fermentation of glucose by rumen ingesta was definitely influenced by the pH of the material, which in turn was found to show considerable fluctuation either on standing at room temperature or after the addition of various acid-forming materials.

The Influence of Glucose Fermentation "in vitro" on the pH of Ruminal Fluid.

While the fistula sheep were being kept on a standard diet of lucerne hay, aliquot samples of ruminal fluid were regularly withdrawn before feeding for the purpose of studying differences in glucose fermentation. The procedure adopted was the same as that described by Quin in article seven of this series, except that in addition to the measurement of the gas volumes, the pH of the material was also studied during the course of fermentation. For this purpose the Beckman Glass Electrode apparatus was used throughout.

In Table 10 results are presented indicating the average range in pH on two successive days taken both before and after the fermentation of glucose (1 c.c. of 20 per cent. solution) by 50 c.c. rumen ingesta under constant movement in a water bath at 39° C.

Table 11.

pH of Rumen Fluid Fermented with Glucose.

Sheep No.	Initial pH.		pH, 10 Minutes after Glucose Fermentation.		pH, 20 Minutes after Glucose Fermentation.		Total Gas Production in 20 Minutes.	
	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
58............	6·91	6·91	7·36	7·30	6·60	6·71	27·5	24·6
32............	6·92	6·89	7·42	7·20	6·69	6·68	20·4	19·5
39............	6·88	6·89	7·26	7·11	6·28	6·70	21·6	26·5
59............	6·90	6·70	7·31	6·99	6·35	6·25	25·0	20·6
63............	6·98	6·82	7·31	7·30	6·81	6·72	24·5	23·5
66............	7·02	6·65	7·48	7·28	6·92	6·69	15·8	20·2
65............	7·10	6·80	7·52	7·39	6·98	6·80	25·6	24·0
43............	7·15	6·81	7·57	7·40	6·99	6·82	25·2	25·0

After fermentation with glucose the ruminal fluid was found to retain its normal light green turbid appearance although the odour was more definitely acid than before. As will be noted from the above table the fermentation of glucose was associated with a primary elevation in the pH of the fluid during the first 10 minutes when all values were found to change over from the slight acidity to slight alkalinity. After a period of 20 minutes, however, the pH was definitely depressed below that of the unfermented material, thus affording evidence of considerable acid formation during the second period of ten minutes.

The Significance of pH in Ruminal Fluid as Related to Glucose Fermentation.

With the object of studying the influence of additional acid on the pH of rumen ingesta, fresh samples of material were repeatedly withdrawn from a single sheep (No. 58). To a series of Erlenmeyer flasks each of which

contained 50 c.c. of freshly strained rumen fluid N. HCl was added in amounts ranging from 1·0 c.c. to 3·5 c.c. and increasing by 0·25 c.c. from flask to flask. After acidification all the samples were fermented with the usual concentration of glucose (0·2 grams) while both the pH and the gas were being recorded at set intervals.

Similarly ruminal fluid was alkalinized by the addition of N. NaOH and the significance of this on pH and on the fermentability of glucose studied in a series of tests, the results of which are presented in Table 12 and the accompanying graph.

TABLE 12.

The Effect of pH Change on Fermentation of Glucose.

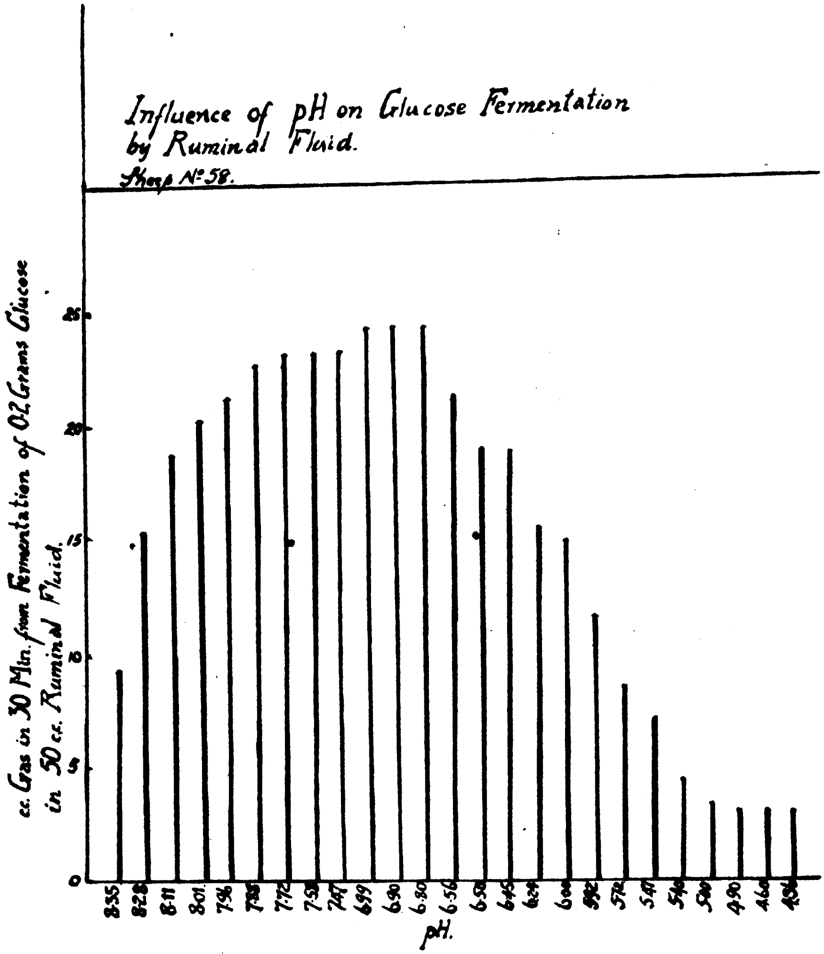
Material.	Initial pH.	After 20 Minutes Fermentation pH.	After 30 Minutes Fermentation pH.	Gas Evolved in 30 Minutes c.c.
Control (50 c.c. ingesta).................	7·02	7·47	6·86	23·0
1·00 c.c. N. HCl added.................	7·02	6·99	6·51	24·5
1·25 c.c. N. HCl added.................	7·02	6·90	6·31	24·5
1·50 c.c. N. HCl added.................	7·02	6·80	6·20	24·4
1·75 c.c. N. HCl added.................	7·00	6·56	5·96	21·5
2·00 c.c. N. HCl added.................	7·00	6·50	5·83	19·2
2·25 c.c. N. HCl added.................	7·00	6·45	5·64	19·0
2·50 c.c. N. HCl added.................	7·00	6·24	5·45	15·5
2·75 c.c. N. HCl added.................	6·96	6·00	5·29	15·3
3·00 c.c. N. HCl added.................	6·96	5·92	5·12	11·4
3·25 c.c. N. HCl added.................	6·96	5·72	5·00	8·6
3·50 c.c. N. HCl added.................	6·96	5·47	4·90	7·2
Control (50 c.c. ingesta).................	7·11	7·72	7·30	23·2
0·15 c.c. N. NaOH added...............	7·11	7·88	7·39	22·8
0·25 c.c. N. NaOH added...............	7·11	7·96	7·46	21·2
0·35 c.c. N. NaOH added...............	7·11	8·01	7·51	20·5
0·80 c.c. N. NaOH added...............	7·01	8·11	7·30	18·9
0·90 c.c. N. NaOH added...............	7·01	8·28	7·30	15·3
1·00 c.c. N. NaOH added...............	7·01	8·35	7·39	8·9
1·50 c.c. N. NaOH added...............	6·78	8·59	8·57	0
2·00 c.c. N. NaOH added...............	6·78	8·89	8·83	0
2·50 c.c. N. NaOH added...............	6·78	9·18	9·20	0

As indicated in the above table and graph (on next page) the following were the main results obtained from these experiments:—

(*a*) Within a range of pH 6·8 to pH 7·8 the "*in vitro*" fermentation of glucose by ruminal ingesta was well maintained at an even level. This was shown by a practically constant rate of gas production within these limits of pH range.

(*b*) The addition of progressively increasing amounts of N. HCl below pH 6·8, resulted in a sharp decline in pH which coincided with a progressive decrease in gas production following glucose fermentation. The end point of this was reached at pH 5·0 to 5·4. With the decrease in gas yield, the motility of the ruminal micro-organisms became depressed.

(c) The addition of N. NaOH to ruminal ingesta beyond pH 7·8 led to a rapid decline in gas production from test fermentations of glucose. Above pH 8·35 no gas at all was produced. This increased alkalinity was accompanied by a darkening of the ruminal fluid which assumed a characteristic musty odour repeatedly noted also in the ingesta withdrawn from animals during starvation. Under these conditions the ruminal organisms likewise displayed decreased motility, the infusoria in particular showing a tendency to die off in large numbers if the medium became too alkaline.



THE EFFECT OF pH WHEN ACID WAS DOSED INTO THE RUMEN OF FISTULA SHEEP.

Following the observation that a change in pH due to acidification of ruminal fluid, affected its fermentative activity, tests were made by dosing equivalent quantities of acid into the rumen.

For this purpose sheep were dosed with dried lucerne leaves suspended in water followed shortly afterwards by 150 c.c. N. HCl through the fistula. This resulted in intense frothing up of the ruminal ingesta. Subsequent to this the pH of freshly drawn samples was recorded at set intervals.

pH of Rumen Ingesta (" in vivo ").

Sheep No.	Initial pH.	After Dosing Leaves pH.	pH after Dosing Acid.			
			25 Minutes.	1·5 Hours.	3·75 Hours.	4·7 Hours.
39............	7·01	6·55	5·90	7·12	7·00	7·00
32............	7·58	7·60	6·52	7·31	—	—

Although the pH of the rumen was temporarily depressed to about pH 5·9 by the dosing of 150 c.c. N. HCl. this did not adversely affect ruminal activity seeing that the animals readily consumed food which was offered shortly afterwards.

Summary.

1. Depending on the carbohydrate content of the diet, the pH of ruminal ingesta in Merino sheep, immediately after withdrawal through rumen fistulae was found to vary between pH 5·5 and 6·8.

2. Whereas the feeding of lucerne hay yielded slightly acid ingesta (pH 6·45 to 6·95), fresh green lucerne (flowering stage), as well as mature veld grass hay produced slightly alkaline conditions varying from pH 7·3 to 7·7.

3. Normally the pH of rumen ingesta in sheep showed slight fluctuation only during the digestion of a single meal, the tendency being towards increased acidity within the first 4 to 6 hours after which it steadily reverts to its previous level.

4. The "*in vitro*" fermentation of glucose by rumen ingesta resulted in a rapid and definite acid production with pH ranging from 6·25 to 7·00.

5. The addition either of N. HCl or of N. NaOH to rumen material showed that it was relatively well buffered between the pH 6·8 to 7·8, whereas beyond this range both on the alkaline and on the acid side the efficiency of this buffering was distinctly reduced.

6. Dosing of 150 c.c. N. HCl directly into the rumen of sheep caused a distinct though transitory depression of pH to 5·9, which however had no obviously detrimental effects on ruminal function.

7. The colour and odour of rumen ingesta was found to be closely correlated with its H.-ion concentration and its ability to ferment glucose.

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Studies on the Alimentary Tract of Merino Sheep in South Africa X.—Notes on the Digestion of Some Sugars in the Rumen of Sheep.*

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In a previous publication from this laboratory (Quin, van der Wath and Myburgh, 1938) in which the subject of ruminant digestion is broadly reviewed, stress is laid upon the great importance of the bacterial degradation of foodstuffs in the rumen as a factor in the conversion of natural products into substances capable of absorption by the animal.

A large part of the natural diet of the sheep is composed of various carbohydrates. While enzymes capable of degrading carbohydrates are elaborated in the more distal portions of the gut, there can be no doubt that very considerate carbohydrate digestion occurs in the rumen and it is indeed probable that this is the main seat of digestion of cellulose, the easily fermented sugars and perhaps to a lesser extent of starch.

It is, therefore, important to study the digestion of various carbohydrates in the rumen with particular reference to the rate of their disappearance and the products obtained.

Without entering into detailed analyses of feeds, it can be stated that the normal diet of the sheep will contain the following substances:—Cellulose, starch, lignin, pentosans, polyuronides (e.g. pectin), sucrose. While sucrose is probably the sugar which occurs naturally in largest quantities, interest also attaches to sugars which can be regarded as probable

* **Editorial Note.**—Miss McAnally spent some time at Onderstepoort during 1939, as a visiting research worker and started an investigation on digestion in ruminants under the guidance of Dr. J. I. Quin, Head of the Department of Physiology. The outbreak of the war and her departure for England prevented her from continuing with her programme of work. Consequently only the preliminary data obtained by her are considered in this report.

Subsequently investigations of the problem at Onderstepoort necessitated a considerable change both in experimental technique and in the interpretation of the findings.

degradation products of the more complex polysaccharides. Chief among these latter sugars is glucose. Owing to the similarity in their constitution respectively to cellulose and to starch, cellobiose and maltose may be added to the list of sugars under review.

The above considerations led to the formulation of the following programme of work. The rate of fermentation of various sugars and the chief products of their breakdown might first be studied. Following upon this study and in the light of the previous results obtained, the more complex problem of the degradation of polysaccharides might be investigated. The results recorded below represent an attempt to carry out the first part of this programme.

Experimental Technique.

One of the major difficulties associated with a study of digestion in the rumen is the daily variation in the nature of the ruminal contents. Thus an animal which can rapidly dispose of dosed sugar on one day may retain traces of it for some hours on the following day. If, therefore, comparison is to be made between fermentations under various conditions, e.g. of different concentrations of sugar or a series of different sugars, great difficulty will be found in interpreting the results of *in vivo* experiments where there is an uncontrolled variable. Whenever possible, therefore, comparisons were made *in vitro*. Thus rumen ingesta was withdrawn from sheep through a rumen fistula according to the method already described (Quin, v. d. Wath, and Myburgh, 1938). The material could then be divided into aliquots and fermentation thus compared under various conditions by exactly similar ingesta

There are plainly objections to working *in vitro* which must always be borne in mind when interpreting results and as far as possible must be obviated by controlling experimental conditions. Thus if the ingesta is kept too long *in vitro* acid formation and putrefaction occur thus rendering the conditions unphysiological. Where fermentation is rapid, as in the case of the sugars *in vitro* experimentation was thought to be more reliable. When, however, fermentation was of longer duration, as with cellulose and starch, it was necessary to use another technique which will be described in a later section.

The rate of gas evolution was found to be a useful index of the rate of fermentation of sugars. This was conveniently observed in a series of 5 c.c. graduated fermentation tubes. As it was impossible to add to these the whole ingesta including solid material, a filtrate obtained by squeezing through muslin was used. A portion of the fermentative agent may in this way be lost, but there should be no error in drawing conclusions when experiments are comparative. Further, a filtrate obtained by squeezing through muslin gave no greater rate of fermentation than material which was allowed to run, under gravity, through muslin. The latter, it may be assumed, would contain less solid material than the former. This filtrate, on centrifugalising, gave an opaque centrifugate which showed very little fermentative power. It would thus appear that the fermentative power resides in the fraction of the filtrate which is thrown down on centrifuging.

The time of withdrawal of ingesta for use *in vitro* work was found to be important. The following figures show the gas evolution from a constant weight of glucose by ingesta withdrawn at the times stated:—

Gas evolved in c.c. by 7·5 c.c. of ingesta filtrate acting upon 0·5 c.c. of 20 per cent. glucose solution.

Time in Minutes (after Addition of Glucose when Gas Reading Taken).	Time of Withdrawal of Ingesta.			
	9.0 a.m.	10.15 a.m.	11.30 a.m.	2.0 p.m.
	c.c.	c.c.	c.c.	c.c.
10	0·4	0·35	0·3	0·2
15	1·3	0·5	0·45	0·45
20	—	—	0·6	0·85
25	—	0·7	0·85	1·05
30	3·65	0·95	—	—
45	4·4	1·35	1·4	1·85

The sheep was fed shortly after the 9.0 a.m. ingesta was withdrawn.

The above results, which have been repeatedly confirmed, show clearly that the fermentative power of the pre-feed ingesta is considerably greater than that of material withdrawn later in the day. The investigation of the reason for this difference constitutes a problem in itself which has not yet been solved. For the purpose of the present work, however, these figures make clear that by using the pre-feed ingesta not only will the optimum conditions for *in vitro* work, i.e. short duration of the experiment, be promoted, but also physiological conditions maintained during which foodstuffs are acted upon by the fasting ingesta.

The pH of the contents of the sheep's rumen is maintained at a fairly constant level, around neutrality, by the buffering action of constituents and more especially by the neutralization of the acid which is formed during fermentation by carbonate secreted in the saliva. Addition of buffer solutions to the ingesta filtrate is unsatisfactory since a sufficient quantity to hold the pH will considerably lessen the fermentative power of the ingesta by dilution.

Allowing the reaction to take place in the presence of excess of calcium carbonate is a suitable expedient when it is possible to stir the whole material periodically. In fermentation tube experiments, however, the calcium carbonate cannot be brought in contact with the material in the top of the tube. Its usefulness is therefore insufficient under these circumstances. The natural buffering power of the ingesta is appreciable and should suffice to hold the pH within physiological limits except when large amounts of acid are being formed. It was thus finally decided to use the ingesta without added buffer. It must, however, be borne in mind in interpreting results that acid formation may affect the course of fermentation in its later stages.

When substrate is added to ingesta in solution it is important to keep the proportion of ingesta to added water as high as possible since, in addition to the loss of fermentative power consequent upon dilution, the added water must first be saturated with gas before any gas evolution is observed.

The concentration of sugar relative to ingesta which is optimum for the study of gas formation can be shown to be 0·1-0·2 gm. of any of the sugars investigated per 8 c.c. of ingesta filtrate. This subject is dealt with more fully below when the fermentation of various sugars is discussed.

The conditions for *in vitro* fermentations which have been applied in the ensuing work may thus be summarised: rumen ingesta was withdrawn by aspiration through the fistula before the morning feed from a sheep which received 400 gm. of lucerne hay twice daily, and was dosed 3 litres of water daily in two portions through the rumen fistula. The ingesta was poured on to muslin and the fluid portion squeezed through. Following this 8 c.c. portions of the filtrate were introduced into the fermentation tubes which were then allowed to stand in an incubator at 37°C until their contents had reached this temperature. The substrate was then added in solution in an amount which was never more than 2 c.c. The ingesta and substrate were then mixed by shaking and the fermentation was observed in the incubator at 37°C.

Formation of Gas as a Measure of the Rate of Fermentation of Various Sugars.

On addition of glucose to ingesta filtrate gas formation sets in rapidly, considerable quantities of gas being formed within the first ten to fifteen minutes. Gas formation proceeds until a point is reached, presumably when the supply of sugar is low or exhausted, when the rate of gas evolution falls off abruptly and though gas continues to be produced the rate is much smaller. By observing the gas evolution in a series of tubes to which different amounts of glucose have been added, it is clearly seen that with increasing glucose content the period of rapid gas evolution is prolonged. The following experiment illustrates this point:—

Gas evolved in c.c. from 8·5 c.c. material containing 7·5 c.c. ingesta filtrate, various volumes of 20 per cent. glucose and the remainder water.

C.c. of 20 Per Cent. Glucose Added.	Incubation Period in Minutes.					
	10	20	30	40	50	60
0·1	0·05	0·05	0·1	0·2	0·3	0·4
0·2	0·2	0·3	0·45	0·65	0·9	1·05
0·3	0·3	1·2	1·8	2·45	2·65	2·8
0·4	0·3	1·2	2·05	2·85	3·15	3·4
0·5	0·25	1·25	2·4	3·1	3·6	3·9
0·6	0·2	1·3	2·4	3·15	3·6	3·85
0·7	·2	1·35	2·45	3·25	3·65	3·85
0·8	0·2	1·35	2·55	3·35	3·7	4·0
0·9	0·2	0·95	2·3	3·05	3·4	3·6
1·0	0·1	1·35	2·65	3·3	3·65	3·8

It appears that while a plentiful supply of sugar is still available, there is a maximum rate of gas evolution which is not exceeded even if the original sugar content is increased in amount. Further, an indication can be seen in the above experiment of a tendency which has been repeatedly demonstrated in a series of similar experiments. If the ratio of weight of glucose to ingesta volume is increased above a value which is approximately 0·2 gm./8 c.c. there is a diminution in the rate of gas evolution.

A comparison was made of the rate of gas evolution from a number of sugars—chief among these were sucrose and maltose, maltose being studied on account of its constitutional similarity to starch. A number of experiments in which these sugars were compared with glucose gave results fully in agreement with that quoted below in which cellobiose as a degradation product of cellulose, and lactose as a sugar wholly foreign to the rumen, were also studied.

Gas evolved in c.c. from 7·5 c.c. ingesta filtrate to which 2·0 c.c. of 8 per cent. sugar was added.

	Incubation Period in Minutes.												
	5	10	15	20	25	30	35	40	45	50	55	60	90
Glucose	0·0	0·35	0·75	1·35	1·75	2·05	2·3	2·6	2·8	2·95	3·2	3·4	4·3
Maltose	0·0	0·0	0·05	0·1	0·2	0·25	0·3	0·3	0·5	0·6	0·7	0·85	2·15
Sucrose	0·0	0·15	0·4	0·65	0·95	1·2	1·5	1·7	1·9	2·1	2·35	2·6	3·5
Cellobiose	0·0	0·0	0·0	0·05	0·2	0·2	0·25	0·4	0·45	0·55	0·65	0·7	1·9
Lactose	0·0	0·0	0·0	0·0	0·05	0·05	0·05	0·05	0·05	0·1	0·2	0·2	0·9

The relationships between sugars is here clearly shown. Glucose is most readily fermented. The fermentation of sucrose does not start so rapidly as that of glucose but soon attains the same rate of gas evolution. In other experiments the gas evolution from glucose and sucrose was more nearly parallel. While sucrose is a natural constituent contained in many of the food plants consumed by sheep, maltose and cellulose are interesting only by virtue of their constitutional relationship to starch and cellulose. It is not surprising, therefore, that these sugars are much less rapidly attacked by the rumen organisms than is sucrose. Cellobiose is perhaps slightly less readily fermented than maltose but since this was the only experiment where cellobiose was observed the difference cannot be regarded as significant. Other experiments where maltose was compared with glucose also indicate that after an initial period of slow fermentation, gas is evolved from maltose quite as rapidly as it is from glucose at the period of most rapid fermentation. It is possible that the initial lag represents the period of hydrolysis of maltose after which fermentation of the resulting glucose takes place normally. Lactose, as might be expected, is very feebly fermented.

Formation of Acid from Glucose by Rumen Ingesta.

For the further investigation of the breakdown products of sugar in the rumen, glucose was chosen for preliminary study as a simple sugar of fundamental importance.

In order to measure the total acid produced in fermentation it was first necessary to test the efficacy as a method of straight forward titration of the fermentation fluid against alkali using phenol phthalein as indicator. Four portions each of 5 c.c. of ingesta filtrate were taken. One was taken as control and to the others were added one, two and three c.c. respectively of standard $\frac{N}{10}$ acetic acid. Each was then titrated against standard $\frac{N}{10}$ caustic potash using phenol phthalein as indicator. By subtraction it was possible to find the alkali neutralised by the 1st, 2nd and 3rd c.c. of added

acetic acid. Thus it was shown that 85 per cent. of the 1st c.c. is recovered, 85 per cent. of the 2nd and 100 per cent of. the 3rd. It is probable that quantitative titration does not occur until carbonate has been destroyed by addition of sufficient acid to neutralise it. Titration of the acidity was therefore performed after the addition to about 7·5 c.c. of ingesta filtrate of 3 c.c. of $\frac{N}{10}$ H_2SO_4. Further it was shown that 2 minutes aeration after the addition of sulphuric acid reduced the titration value owing to the removal of CO_2. By adopting these two methods it was thus possible to obtain figures for variations in total organic acid content due to fermentation of sugar.

In all cases examined it was found that total acidity rose rapidly to a high level within ten to fifteen minutes after addition of glucose and that further increase of acid was not then appreciable. Comparison of the rate of acid and of gas production showed that gas evolution continues after the acidity has reached its maximum level.

The following two series of results (A and B) are typical and serve to illustrate the above conclusion:—

> Fermentation tubes containing 7·5 c.c. ingesta filtrate plus 0·5 c.c. of 20 per cent. glucose were emptied after the time intervals stated and after the gas volume had been read off. Following this the acid was titrated according to the method described above.

		Time in Minutes.								
		5	10	15	20	25	30	35	40	45
Gas evolved in c.c.	A.	0·3	1·45	3·1	4·25	4·4	4·7	4·95	4·5	4·6
	B.	0·25	0·7	2·8	3·8	4·4	4·6	4·6	3·95	4·2
Acid in c.c. $\frac{N}{10}$ in excess of control	A.	—	3·45	3·35	3·85	4·05	4·05	3·9	4·0	4·4
	B.	2·1	1·9	3·55	3·65	3·85	3·95	4·45	4·25	4·25

It would thus appear that considerable acid production precedes gas evolution. As an explanation of this fact it may be suggested that oxidation of glucose with the formation of hydrxoy acid of high molecular weight may precede true fermentation with the production of gas and small molecule acids.

In order to test this hypothesis it was necessary to compare the rate of total acid production with that of the volatile and ether soluble non-volatile acids. In all cases the volatile acid content of fermentation fluid was by no means so dependent upon time of incubation as was that of total acids. The curves for volatile acids against time were very irregular in form though there was a general tendency for volatile acid to increase after the first 15-20 minutes.

30 c.c. of ingesta filtrate (A) and 30 c.c. of ingesta filtrate plus 2 c.c. of 20 per cent. glucose (B) were incubated at 37° C. for 15 minutes. Some aluminium sulphate was then added to each and the clear liquid separated from the precipitation protein by centrifuging. The clear liquid together with washings was in each case made up to 100 c.c. 50 c.c. was taken from

each, excess sulphuric acid added and the volatile acids distilled with steam until 50 c.c. of distillate neutralised less than 1·0 c.c. of $\frac{N}{10}$ alkali. The residual liquid was thoroughly extracted with ether and the ether extract titrated with $\frac{N}{10}$ alkali. Following this 25 c.c. was taken from (A) and from (B), $\frac{N}{10}$ alkali was added to neutralise exactly and the material was evaporated to dryness. The resulting salts of organic acids were then taken up in water, the solution filtered and then evaporated to dryness and the residue ignited. The ash will contain carbonates in equivalent amount to the organic acids and their salts which were originally present. By titrating this ash with standard acid, therefore, their amount can be determined.

Cubic Centimetres $\frac{N}{10}$ Alkali Equivalent to:—

	1	2	3	4
Total organic radicals..............................(A.)	25·6	19·8	20·4	16·2
Total organic radicals..............................(B.)	33·0	28·4	29·0	30·4
Volatile acids..............................(A.)	12·9	13·6	16·3	11·9
Volatile acids..............................(B.)	9·6	9·0	13·7	12·7
Ether soluble non-volatile acids..............................(A.)	1·2	3·7	5·9	3·8
Ether soluble non-volatile acids..............................(B.)	1·3	1·4	8·1	3·4

By comparing the (A) and (B) pairs in this table of results it can readily be seen that whereas, in a 15 minute incubation, there is a marked increase in total organic acid radicals, this is accounted for neither by volatile acids nor by ether soluble non-volatile acids.

Further analysis is required to determine the nature of this unidentified acid fraction. Whatever its nature may be it is probable that it represents a stage only in the breakdown of sugar to gas and acids of small molecular size. Demonstration, however, of the existence in the rumen for any considerable length of time of, for instance, hydroxy acids of three, four, five or six carbon atoms would be of considerable interest from the point of view of the nutrition of the animal.

Digestion of Polysaccharides in the Rumen.

Owing to the relative slowness of attack upon polysaccharides such as starch and cellulose by the rumen organisms, the methods described for the study of sugar fermentation are unsuitable.

A successful method has, however, been devised for the study of the disappearance of insoluble materials *in vivo*. This has so far been applied to cellulose only.

Suitable aliquot quantities of cellulose (mashed filter paper) were weighed out accurately on to a series of 2½ inch squares made of some fine natural silk material. The squares were previously moistened to prevent fine particles passing through the pores of the material. The edges were brought together and bound round with silk. About six or eight such bags were lightly bound together and suspended by a silk cord in the rumen of a sheep having a large (5 cm. in diameter) rumen fistula. After suitable periods the whole bunch was removed, a single bag cut off and the rest returned to the rumen.

The residual material in the bag was then thoroughly washed with a jet of water, using the silk as a filter, then dried on a water bath and weighed.

From the few results thus far obtained it would appear that digestion of cellulose, under the circumstances of these experiments does not start until during the course of the second day. In each case it was found that digestion of the cellulose was appreciable between the 20th and 26th hours. Further work is being carried out along these lines.

Summary.

1. Studies were undertaken on the disintegration of various carbohydrates (sugars and cellulose) in the rumen of sheep. For this purpose were utilised adult merino sheep with permanent fistulae in the rumen.

2. With the animals on standardized diets, ruminal ingesta was periodically withdrawn by aspiration through the fistula. This material was then filtered through fine muslin and fermentative activity of the filtrate determined by measuring the volumes of gas evolved in fermentation tubes following the addition of different concentrations of sugars.

3. Results obtained show the extreme rapidity with which sugar is fermented by rumen ingesta, the rate and degree of fermentation depending on (*a*) type of sugar used, (*b*) its concentration in the tubes, and (*c*) the nature of the ingesta and the time of its withdrawal.

4. Accompanying the evolution of gas, there is a rapid rise in total acids within the tubes. This, however, cannot be wholly accounted for either as volatile acids or as ether soluble non-volatile acids, thus necessitating further investigation,

5. Methods are described for determining the rate of disappearance of cellulose within the rumen, through the enclosure of weighed amounts of cellulose in thin silk bags and the suspension of these through the rumen fistula.

Further work along these lines is still in progress.

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The Effects of Diethyl-Stilboestrol and Pregnant Mare Serum on the Oestrous Cycle of Merino Ewes.

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As is the case in other sheep breeding countries, it is a common experience in South Africa that the sexual activity of merino ewes may be suppressed for a period of several months each year. Where this period is unduly prolonged, it may, therefore, interfere with normal breeding operations.

In an extended series of investigations, Quinlan and Mare (1931), Roux (1936), Marais (1936), and more recently Quinlan, Steyn and de Vos (1941) found the anoestrous period in merino ewes under South African conditions to last approximately from September to December, i.e., during the first half of the summer season. In Europe, similar findings of anoestrus during the summer months have been recorded by several authors. According to Hammond, Hammond & Parkes (1942), the ovaries of such ewes are, however, not entirely quiescent during the anoestrous period seeing that enlarged Graafian follicles may at times be found in them and which in isolated cases result in actual ovulation, although not accompanied by any signs of clinical heat. This phenomenon of ovulation associated with so-called "silent heat" has been repeatedly observed by different workers, although its genesis as well as that of anoestrus itself still awaits final elucidation.

Concerning the aetiology of seasonal anoestrus in ewes it has been suggested (Parkes and Hammond, 1940) that this might be associated with a temporary insufficiency of gonadotrophic and follicular hormones. The resumption of normal pituitary and ovarian function in turn appears to be related with the nutritional state of the animals, evidence of which is afforded by the stimulating effect of "flushing" in contrast to the prolonged anoestrus displayed during periods of drought and poor feeding. According to van der Horst and Gillman (1942), anoestrus, the onset of which is a relatively acute process, inhibits the further formation of gonadotrophic hormones in the pituitary.

Apart from the possibility of these temporary fluctuations in the endocrine balance of the animal body, periodic changes in the reactivity of the reproductive organs, even to normal hormone stimulation, may in themselves be found to contribute towards the onset of the anoestrous state. In this connection the rôle exerted by nutritional and climatic factors, both on the level of hormone production as well as on the responses shown by the genitalia, constitutes an aspect of sex physiology requiring further investigations of a more fundamental nature.

With the object of inducing normal oestrous cycles during periods of anoestrus, hormone treatment in a variety of forms has, within recent years, been undertaken in different species of animals. As in other branches of endocrine therapy, the response elicited by such treatment has been found to vary with the nature and origin of the hormone used, with its dosage and methods af administration, and finally also with the physiological state of the body and the individuality of the animal.

In all this work two essentially different types of compounds have been employed throughout. These include (*a*) the *gonadotrophic hormones* as derived primarily from the anterior lobe of the pituitary, and in addition those encountered either in the serum of pregnant mares or in human pregnancy urine. According to present knowledge the two gonadotrophic hormones from the anterior lobe of the pituitary, viz., follicle stimulating and luteinizing respectively initiate and control ovarian rhythm and ovulation. In turn the two secondary hormones elaborated within the ovary, viz., oestradiol (formerly also known as folliculin) and progesterone, as derived from the corpus luteum, are known to be responsible for the various clinical phenomena associated with heat and the rest of the oestrous cycle, and thus also for the development and maintenance of the uterine and mammary tissues. (*b*) *Oestrogenic compounds* derived as naturally occurring hormones either from the ovaries, placenta, or from human pregnancy urine or alternatively as chemically synthesised compounds amongst which the highly potent eostrogen diethyl-stilboestrol has, through recent investigations, gained much prominence. Apart from their direct stimulating effects on the uterus, vagina and mammary glands, various oestrogenic compounds administered in adequate amount have been shown to exert a peculiar trigger-like action on the anterior lobe of the pituitary itself, a phenomenon which is as yet not fully understood [Steinach, Stäheli, Grüter (1934), Folley and Malpress (1941)]. According to Walpole (1941), the results of this so-called " oestrogen shock " depends upon the release of gonadotrophin from the anterior pituitary subsequent to a primary inhibition of this organ. Whatever the exact nature of the mechanism may be, evidence is accumulating to show that the judicious use of certain oestrogenic compounds are as effective in initiating ovulation and oestrus as are the gonadotrophic hormones themselves.

Seeing that the costs of production of highly potent stilboestrol is lower than that of the pituitary gonadotrophic hormones, the indications are that this synthetic compound will be extensively used in the treatment of certain types of functional derangement of the reproductive organs and especially in the initiation of the oestrous cycle in otherwise anoestrous animals. Accordingly a very considerable volume of literature has already been published concerning the action and uses of stilboestrol. Thus Montgomerie and Brownlee (1941) and Brownlee, Gould and Stuart (1942) record full oestrus in anoestrous heifers and cows following a single injection of 10-20 mg. of stilboestrol dipropionate. This is followed by a regular succession of normal oestrous cycles. Likewise Anderson and Bugg (1942) found that six anoestrous heifers were promptly brought into oestrus within 48 hours following the injection of 15 mg. stilboestrol dipropionate. Service at this stage, however, failed to induce pregnancy. Hammond, Hammond and Parkes (1942) record ovulation in the majority of anoestrous ewes following the injection of 0·5 to 1·0 mg. stilboestrol dipropionate. Both oral and vaginal administration of this compound failed, however, to produce these effects.

The results obtained with stilboestrol are essentially similar to those recorded from the use of oestradiol. Thus Steinach, Stäheli, and Grüter (1934) and Folley and Malpress (1941) were able to initiate regular oestrous cycles associated with fertile ovulation by the use of oestradiol (dipropionate or monobenzoate) on anoestrous heifers and cows. Likewise McKenzie and Terrill (1937) and Anderson (1938) record the onset of oestrus in the majority of anoestrous ewes treated with oestradiol benzoate.

Apart from the stimulating influence of stilboestrol on the genitalia, Folley and Young (1941), Lewis and Turner (1940) and Walker and Stanley (1941) noted a remarkable degree of mammary development in heifers and goats which in certain cases even resulted in copious lactation. According to Lipschütz and Vargas (1941) the exceptional activity shown by stilboestrol is probably associated with a greater resistance to inactivation in the animal body as compared with the natural oestrogens. From the findings of Sealy and Sondern (1941) the naturally occurring hormones, oestrone, oestradiol and oestriol were $\frac{1}{16}$, $\frac{1}{4}$, and $\frac{1}{40}$ respectively as active as stilboestrol when assayed by subcutaneous injection into test animals.

The Reaction of Anoestrous Merino Ewes to Stilboestrol.

In order to ascertain the effect of stilboestrol on merino ewes during the period of anoestrus, a series of experiments were undertaken at Onderstepoort in which varying amounts of stilboestrol were administered to a total of 43 full grown animals during the months of November and December. The complete absence of clinical oestrus amongst these animals, immediately prior to treatment as well as for several months preceding the experiments, was established by regular daily admission (morning and evening) of "teaser" rams amongst the ewes. Detailed records were kept of the oestrous response of individual animals. The brand of stilboestrol used was that supplied by Messrs. Boots, Nottingham. In all the tests, except one, the material was dissolved in oil and administered by deep intramuscular injection, the dosage ranging between 1 mg. and 5 mg. stilboestrol per sheep.

The following table indicates the results obtained from the different tests.

No. of Animals Used.	Dose of Stilboestrol.	Reaction Displayed.
12	5 mg. injected............	3 in full oestrus in 48 hours and served. Remaining 9—Negative.
11	5 mg. dosed by mouth.....	All negative.
13	2·5 mg. injected..........	3 in full oestrus in 48 hours and served. 5 in weak, doubtful oestrus, not served. 5 remained negative.
4	1·5 mg. injected..........	1 in full oestrus in 24 hours and served. 1 in oestrus after 4 days. 2 negative.
3	1 mg. injected............	3 in weak doubtful oestrus in 24–48 hours—None served.

As will be noted from the above results 8 out of the 43 ewes treated with stilboestrol came into full oestrus, while a further 8 ewes displayed poor and doubtful signs of heat.

In order to ascertain the effects produced on the ovaries and uterus, two ewes which came into full oestrus 48 hours after the injection of 5 mg. stilboestrol were laparotomized on the 6th day following injection. In each case young corpora lutea were detected in the ovaries thus affording evidence of recent ovulation. The uterus in both ewes was pale and inactive. A further two ewes injected with 2·5 mg. intramuscularly, which had failed to display any signs of oestrus, were likewise lapafotomized six days after injection. In one case there was a mature follicle (5 mm. diameter) present in one of the ovaries while the uterus appeared enlarged, turgid and hyperaemic. In the other ewe one of the ovaries showed a young corpus luteum in addition to an apparently mature follicle. Again the uterus appeared enlarged and hyperaemic. From the laparotomies thus performed it was evident that where oestrus was induced by stilboestrol, this was associated with ovulation and corpus luteum formation. Likewise there was definite evidence of follicle stimulation, ovulation, corpus luteum production and uterine reaction in two ewes despite the absence of any signs of clinical oestrus. These must be regarded as typical cases of ovulation with "silent heat" the aetiology of which as indicated before still awaits elucidation.

In addition to the above ewes treated during the anoestrous period, a further seven ewes were injected with stilboestrol (1 mg.) in April and May (i.e. during the height of the sexual season), in each case on the 8th day following normally occurring oestrus. Of these ewes only one came into full oestrus after 24 hours. This animal, together with two of the other ewes which failed to show oestrus, were laparotomized within 48 hours after injection. In all three animals there was evidence of recent ovulation as revealed by the presence of fresh corpora lutea together with definite enlargement and hyperaemia of the uterus.

As in the case of the anoestrous ewes, the results produced by stilboestrol on these seven normal ewes in dioestrus clearly indicate that ovulation is promptly induced by doses of stilboestrol as small as 1 mg. injected intramuscularly although in the majority of cases this is not accompanied by any signs of clinical oestrus. Similar experiences have been recorded by Parkes and Hammond (1940) with the use of horse pituitary extracts on anoestrous ewes and also by Van Aswegen and Quinlan (unpublished data) on the effects of prolan.

THE EFFECTS OF STILBOESTROL ON SPAYED EWES.

In order to test out the direct action of stilboestrol on the uterus and vagina, i.e., without the intermediation of any ovarian effects, five adult merino ewes were spayed in December, 1940. After a lapse of 12 months all the animals were injected with stilboestrol at varying intervals. The results of these tests are depicted in the following table:—

Time of Injection after Spaying.	Stilboestrol Injected.	Results.
12 months..........	5 mg............	All 5 ewes in full oestrus in 24–48 hours allowing repeated service for further 2–3 days.
16 months..........	1 mg............	All 5 ewes in full oestrus in 24–48 hours allowing repeated service for further 2–3 days.
17 months..........	0·5 gm..........	Two ewes in full oestrus in 48 hours. Three ewes remained negative.

A subsequent injection of 0·3 mg. into the two ewes which gave a positive response with 0·5 mg. likewise induced full oestrus in both animals within 48 hours. The remaining three ewes which failed to respond to 0·5 mg. were later on injected with 0·75 mg. with the result that only one came into oestrus.

This experiment conducted on spayed ewes reveals the interesting finding that despite the loss of the ovaries 12 months previously, the remaining parts of the genital system showed an even greater degree of sensitivity to minute doses of stilboestrol than was the case in normal ewes. Thus doses of 1 mg. stilboestrol (and in some cases even less) promptly induced oestrus in all the spayed animals whereas in normal anoestrous ewes only a small percentage (8 out of 43) came into heat even with larger doses of stilboestrol, and despite the occurrence of ovulation which was regularly noted in laparotomized animals. In addition to the strong oestrogenic effects produced on the genitalia of spayed ewes, stilboestrol was also found to cause rapid development and turgidity of the mammary glands accompanied by actual milk secretion in two of the ewes. This confirms the findings of various other investigators previously referred to.

Reaction of Anoestrous Ewes to Injections of Pregnant Mare Serum.

Repeated investigations have shown that the blood serum of pregnant mares collected at a certain stage of pregnancy exerts powerful gonadotrophic effects when injected into non-pregnant females of different species. According to Day and Rowlands (1940), significant concentrations of gonadotrophic hormone appear in the blood of the mare only after 30-47 days of pregnancy. Subsequently it reaches its highest titre at 60-75 days, only to be followed by the disappearance of this hormone approximately 110 days after the onset of pregnancy. With the use of single doses of active serum, Cole and Miller (1939) were able to induce ovulation in anoestrous ewes which was unaccompanied, however, by any signs of oestrus. A further injection 16 days later was found to provoke both ovulation and oestrus. Bell, Casida, Bohstedt and Darlow (1941) similarly observed ovulation without oestrus in ewes treated with such serum. Cameron (1942) found the serum more effective on mares than on cows. From 11 anoestrous ewes each injected with 300-400 R.U. pregnant mare serum, 5 animals ovulated after 30-35 hours, although only one came into heat (McKenzie and Terrill, 1937). Hammond, Hammond and Parkes (1942) noted that all out of 11 anoestrous ewes killed 53 hours after the injection of 200-2,000 I. U. of pregnant mare serum had ovulated. These authors conclude that both anterior pituitary extract and pregnant mare serum evoke oestrus only in the presence of a regressing corpus luteum, while in the absence of this body, ovulation is not accompanied by heat. An active corpus luteum on the other hand supresses both ovulation and heat.

With the object of studying the effects of pregnant mare serum on merino ewes under South African conditions, four mares were bled on the 90th day of pregnancy and equal quantities of the different sera subsequently mixed. This serum preserved with merthiolate (1 : 100,000) was used throughout in all the tests, which were conducted on a total of 110 adult merino ewes during the period October to December. All animals were in anoestrus during the whole of this period as shown by regular daily testing (morning and evening) with vasectomised rams. The following data was collected in the various experiments following subcutaneous injection of pregnant mare serum.

Experiment No. 1.—16 ewes injected 100 c.c. serum containing 100 mouse units per c.c., each on October 8th.

Result—Rams attracted by all ewes within 24-48 hours. Oestrus, however, incomplete as no ewe allowed service. Eight ewes slaughtered 72 hours after injection. In all cases ovaries found to contain enlarged cystic follicles up to 1 cm. in diameter although no ovulation recorded. Uterus in all animals only slightly turgid and vascularized although vulva definitely hyperaemic. Remaining 8 ewes came into normal oestrus following January.

Experiment No. 2.—10 Ewes injected 10 c.c. serum (1,000 m.u.) each on October 21st.

Result.—No definite signs of oestrus. Four ewes slaughtered 72 hours after injection. Ovaries in all cases enlarged and cystic but as in experiment No. 1, no ruptured follicles noted.

Experiment No. 3.—8 Ewes injected 0·5 c.c. serum (50 m.u.) in November.

Result—One ewe promptly in oestrus within 24 hours. Remaining 7 ewes showed no signs of oestrus, but vulva hyperaemic. From four of these slaughtered after 72 hours, three had definitely ovulated some hours previously, while the fourth animal showed mature follicle on point of rupture in one ovary. Three ewes not slaughtered came into normal oestrus in January.

Experiment No. 4.—35 Ewes injected 0·25 c.c. serum (25 m.u.) October 21st.

Result.—Although 4 ewes attracted the teaser rams, not one came into full oestrus. All ewes artificially inseminated 48 hours and again 72 hours after injection. None, however, showed pregnancy as all ewes came into oestrus during following January and February.

Experiment No. 5.—Late in anoestrous season (11th December) 41 ewes injected 0·25 c.c. serum (25 m.u.) while 30 untreated ewes remained as controls.

Result.—(*a*) 8 Treated ewes came into full clinical oestrus in 24-48 hours, 2 ewes in 5 days and 4 ewes in 8 days following serum injection, while all control animals failed to show oestrus. All treated ewes artificially inseminated 48 hours and again 72 hours following injection. Of these, only 4 ewes which showed oestrus within 24 hours became pregnant and lambed normally.

(*b*) 12 Treated ewes attracted rams but would not mate. External genitalia red and swollen. Together with control ewes these animals came into normal oestrus in January.

(*c*) 15 Treated ewes showed *no* sign of oestrus. Of these 9 were laparotomized within 3-5 days following injection. Fresh corpora lutea 24-48 hours old were found in the ovaries of 6 ewes showing that they had all ovulated very recently. In three cases the uterus appeared swollen and hyperaemic and the adnexia thickened and vascular while in the other three ewes only slight uterine changes were noticeable. In another one of the 9

ewes treated with serum a mature follicle was found in one of the ovaries, the uterus being enlarged and flaccid. The ovaries in the remaining two ewes were found to be small with the presence of atretic follicles in them.

Discussion and Conclusions.

There is ample evidence from the literature cited, that the various gonadotrophic hormones as contained in anterior pituitary extracts, pregnant mare serum, and to a lesser extent in human pregnancy urine, possess very definite follicle stimulating properties on the ovaries of various classes of animals, while in the anoestrous state. Similar results are achieved by the use of naturally occurring, as well as by synthetically prepared oestrogens, which through their effects exerted on the anterior lobe of the pituitary, likewise result in follicle stimulation. In the majority of cases this is associated with ovulation. Up to this point, our own data obtained from anoestrous merino ewes treated either with stilboestrol or with pregnant mare serum fully confirm the findings of other workers. Thus of all the treated ewes which were subsequently slaughtered or laparotomized, examination of the ovaries revealed either very recent ovulation in the majority of cases or the presence of mature follicles on the point of rupture.

With regard to the induction of full clinical oestrus and the willingness to mate, the results of the different investigations are, however, far more at variance. This is especially the case in ewes showing a well-defined seasonal anoestrus. In cows and heifers on the other hand where this is less evident, information thus far available tends to show that induced ovulation is more regularly accompanied by the onset of oestrus and the initiation of normal cycles than is the case in ewes. Thus, despite the high percentage of ovulation evoked amongst our experimental ewes, only 8 out of 43 treated with stilboestrol and 9 out of 110 which received pregnant mare serum came into heat sufficiently evident as to allow service. In contrast to this, the oestrogenic effects of stilboestrol in doses of 1 mg. intramuscularly were most decisive in the case of spayed merino ewes. Not only were all 5 animals used in the test repeatedly brought into heat after the injection of stilboestrol, but in every instance the onset of oestrus was both prompt, occurring usually within 24 hours, and fully sustained for a further period of 2-3 days, i.e., considerably longer than oestrus normally observed in ewes.

This finding affords strong evidence that the complete withdrawal of ovarian influences as achieved through spaying, provokes a definite increase in the sensitivity of the remaining genital tract to oestrogen subsequently administered. Conversely the oestrous response shown by a large percentage of anoestrous ewes was found to be completely absent when tested either with oestrogen (stilboestrol) or gonadotrophin (pregnant mare serum). No satisfactory explanation can as yet be offered for the wide fluctuations observed in the reaction of the uterus and vagina of ewes, which in the final instance forms the deciding factor in the onset of clinical oestrus. One possibility, however, which merits further investigation is the production of anti-hormone following upon repeated stimulation of the genital tract by oestrogen periodically released in the ovaries. According to this concept the phenomenon of seasonal anoestrus in ewes would then be explained not only on the basis of decreased gonadotrophic activity of the anterior pituitary as at present accepted, but equally as much on a temporary though cumulative resistance of the uterus to the full effects normally

exerted upon it by oestrogen. To what extent this fluctuation in uterine response is further influenced by such factors as age, breed, nutrition, climate and season is a matter awaiting more fundamental investigation. There is evidence to suggest, however, that the divergent results achieved by different investigators from the use of these hormones on anoestrous animals and especially on ewes, may be ascribed largely to differences in the physiological state of the individual animal at the time of treatment.

In this connection special reference should be made of the importance of the stage of anoestrus at which the investigations are conducted, seeing that different responses may be expected from animals during the early phase as contrasted to the end of the anoestrous period.

The observation repeatedly made, that ovulation as such could be readily induced by a variety of hormones during anoestrus, affords proof that the sensitivity of the ovaries to gonadotrophic influences is less effected than that of the uterus and vagina to oestrogen, whether this be naturally produced within the ovary or artificially injected. Moreover, the fact as pointed out by Hammond, Hammond and Parkes (1942) that the ovaries of anoestrous animals are not entirely quiescent during this period, indicates that gonadotrophic activity in the anterior pituitary is not completely suppressed, hence the explanation for the enlarged follicles which at times result in ovulation, although unassociated with heat (silent heat).

Concerning the influence of environmental factors on the anoestrous state, Zawadowsky and Margulis (1939), cited by the above authors, found that where a proportion of ewes came into heat spontaneously during a favourable year, their number could be considerably increased by a single injection of pregnant mare serum, while a second injection 16 days later evoked heat in 25 per cent. of the animals. The following year, however, when the ewes were in poor condition after a hard winter, and few came into heat spontaneously, neither one nor two injections of pregnant mare serum resulted in more than 1 per cent. of the animals taking the ram. These findings, in common with those of other workers, clearly indicate that the results to be expected from hormone therapy in the field of animal breeding, depend as much on the physiological conditions within the animal body at the time of treatment as on the nature and potency of the hormones administered. In this connection the degree of responsiveness shown by the anterior pituitary, the ovaries and particularly also by the female genital tube, forms the main deciding factors.

From the observations made on normal ewes as well as on spayed ewes following treatment with stilboestrol and pregnant mare serum, evidence is presented showing that seasonal anoestrus in merino ewes is closely associated with a decreased physiological response of the uterus and vagina to oestrogens.

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Depletion of Substantial Vitamin A - Reserves in Growing Rats.

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RATS require for normal growth, health and reproduction, a certain minimum level of vitamin A. This was found to be 18-22 International Units (I.U.) of vitamin A per Kgm. body weight, by Gross and Guilbert (1939). When rats receive vitamin A in excess of these levels, they are able to store vitamin A in the liver. Should the animals receive a ration deficient in this factor, the reserves are drawn upon to maintain normal growth, appetite and health.

In cattle, Riggs (1940) has found that the time required for total elimination of vitamin A from the livers was from 56 to 178 days, depending on the age of the animals (ages varying from 3 to 16 months) and also depending upon the levels of the reserves of vitamin A stored. Davies and Moore (1935, 1937) working with rats, came to the conclusion that when high reserves were built up due to massive doses of vitamin A given in the feed, the depletion is at first rapid but eventually the reserves reach a level when very small demands thereon are made, thus enabling the rats to reach maturity without suffering from a vitamin A deficiency.

In a later publication, Moore (1940) showed that vitamin E-deficiency in conjunction with vitamin A-deficiency, caused a more rapid elimination of the vitamin A-reserves. In the present work a ration was given which lacked both vitamin A and vitamin E and a study was initiated to determine the rate of vitamin A-elimination from the livers, when rats had previously stored about 1,200 I.U. of vitamin A. The ration was the same as the one used in other vitamin A-deficient work at this Institute (1943).

The percentage composition of the ration was:—

Modified Bacharach (1931) Ration	Casein (ether and alcohol extracted) ...	18·5	gm.
	Dextrinized Starch	50·0	gm.
	Fat	12·3	gm.
	Yeast	10·0	gm.
	Canesugar	0·08	gm.
	Salts (Kellermann—modified Steenbock)	5·12	gm.

The ration was freshly prepared each week and fed to the rats *ad lib.* The rats were divided into three groups: One group (Group A, control) was given the ration fortified with cod liver oil (±2 per cent.). An equal number of rats (16) with the same average body weight (viz. 103 grams)

was fed the deficient ration (Group B). A third group (Group C) was also fed the deficient ration and was reserved for the time when livers would be depleted; in other words, the rats of this group would serve as examples of vitamin A-deficiency, apart from body weight. All the rats in these groups were males of nearly the same age (from 5 to 6 weeks old).

During the pre-experimental period the rats had access to a full, well-balanced ration (rat stock ration) as used by this Institute for general purposes of breeding, etc. During this time (up to 6 weeks) a reserve of about 1,200 International Units had been built up. (The stock ration contained cod liver oil as well as crushed yellow maize, which served as sources of vitamin A.)

Two rats were slaughtered in each group (A and B) at intervals of four weeks to obtain the levels of vitamin A in the livers by chemical method. The method of Moore (1930) was employed and the evaluation of the extracts was done by the Carr-Price test in the tintometer. The liver assays served as indication of the rate of depletion of vitamin A. All the rats were housed in a proper rathouse, well ventilated and heat controlled. Fresh feed and water were given *ad lib.* daily. At the stage when depletion was complete, attention was given to the third group of rats. Records of the symptoms of deficiency as they occurred were made.

Conclusions and Discussion.

It was seen that the depletion of the reserves in the deficient group was rapid (Table I). On the average there was a decrease of more than 50 per cent. of the total reserves within the first four to eight weeks. A less rapid rate of decrease followed in the next 8 weeks but by the 20th week the average liver reserves showed a decrease of 90 per cent. of the initial reserves. After 24 weeks the livers of the rats slaughtered were completely depleted of vitamin A and at the expiration of a further 4 weeks, at the 28th week, another set of rats were slaughtered and again the livers showed no vitamin A. It was thus conclusive that the livers were totally depleted. At the 26th week, symptoms of vitamin A deficiency were observed in the deficient group (the reserve group of rats). These symptoms are described in detail.

Table 1.—*Average Vitamin A Reserves of the Livers of Rats.*

Control Group.					Vitamin A Depletion Group.				
Experimental Period.	Body Weight. Grams.	Liver Wht. Grams.	Whole Liver I.U. Vit. A.	Per gram. I.U. Vit. A.	Experimental Period.	Body Weight. Grams.	Liver Wht. Grams.	Whole Liver I.U. Vit. A.	Per Gram I.U. Vit. A.
Pre-experimental	109	7·0	1,120	162	Pre-experimental	101	5·5	1,123	145
After 4 weeks...	184	7·8	858	109	After 4 weeks...	165	8·5	424	52
After 8 weeks...	220	9·3	1,008	108	After 8 weeks...	207	9·5	504	53
After 12 weeks..	241	10·0	1,764	176	After 12 weeks..	228	8·0	319	40
After 16 weeks..	267	11·2	1,846	166	After 16 weeks..	241	8·4	263	31
After 20 weeks..	280	12·0	1,607	136	After 20 weeks..	275	11·2	124	11
After 24 weeks..	348	15·7	1,967	125	After 24 weeks..	268	10·4	0	0
After 28 weeks..	325	12·3	1,323	108	After 28 weeks..	276	7·8	0	0

In the control group the liver reserves showed after the first 4 weeks a decrease due mainly to a change in the diet. Thereafter an average increase in vitamin A reserves was noted for the following weeks, with a peak average value of 1967 International Units in the whole liver at the 24th week. At this stage the rats registered the highest body weights, whereas in the deficient group B the body weights of the individual rats had become constant at various earlier periods. The body weights were recorded at weekly intervals and the average body weight per group is given in Table 1, as well as the average liver reserves for the two groups.

From 20 to 24 weeks were necessary to deplete the liver reserves of growing rats fed a vitamin A-deficient ration when a liver reserve of 1,200 I.U. of vitamin A had been found in the initial stage. In another paper (1943) the author used younger rats, at weaning stage, with an average reserve of only 10 I.U. vitamin A. The depletion period of this small reserve was 43—52 days for two days for two sets of rats, intended for biological tests with vitamin A.

Symptoms of Vitamin A-deficiency (Group C).

(a) *General Symptoms.*

At more or less the same time 3 rats of the Group C showed symptoms of vitamin A-deficiency. The early symptoms were observed in the eyes. Sensitivity towards light was obvious; the rats kept their eyes partly closed. The eyes on examination appeared dull compared with the controls. Dry, flaky discharge on the upper eyelids (keratinization) as well as deterioration of the eye glands were observed. Rats became progressively listless and lethargic, lying down most of the time, curled up with the head hidden from light. The appetite became poor and afterwards was completely lost. Food was left entirely untouched.

The symptoms following on the above were observed as the disease progressed, and can be considered as later, advanced symptoms. The mucous membrane of the lower jaw was tainted red and later bloody, especially the gums of the lower incisors. The mucous membrane of the nasal chambers was pale and dry, but later a catarrhal discharge, brown and sticky, was seen. Due to sensitivity towards extreme temperatures (heat) the breathing became laboured (not evident in control group) and in advanced stages, inhalations and exhalations were irregular with gasps or panting at intervals. The movement of the rats was generally affected due to degeneration of the muscles; at first difficulty in walking, crouching and dragging of the body were observed. Later the hind legs became partly paralysed (paresis) and the animals sprawled when resting; very often lameness in one limb or more was observed. The nervous system was also impaired. Rats twitched, shivered and shirked handling even though they were normally tame and used to handling. In extreme cases diarrhoea set in and in one case deafness was noted.

Schmidt (1941) described symptoms observed in ruminants, also Hart (1940) for cattle, horses, pigs and sheep, which are very similar to those seen by the author in the rats. Photographs are given as illustrations here.

(b) *Observations on Individual Rats in Group C.*

Rat No. 1.—Body weight 175 grams.

In one week after depletion the following successive symptoms were observed: The rat very gradually became worse and died soon afterwards; light sensitivity; a discharge of the eyes; loss of appetite and general listlessness; breathing became difficult with gasps at intervals; nasal discharge which irritated the animal considerably. On the last day, before death, the rat was very nervous, had convulsions of short duration (spasms). Diarrhoea had set in and the eyes appeared so badly affected that blindness was apparent. Body weight of rat (wasting had taken place rapidly) was 175 gm. compared with 296 gm. of one of the controls of the same age.

Rat No. 2.—Body weight 180 grams.

General symptoms as described were evident. The photograph clearly shows the rat in a sprawling attitude due to partial paralysis of the hind legs.

Curative treatment was practiced, at first by dosing small doses of fish liver oil (diluted down with olive oil to appropriate strengths). The dosing was done orally by pipette. As the rat improved on the third day vitaminized food was given. The appetite of the animal was quickly restored. The eyes became brighter and were soon apparently normal. Vitality improved. After ten days curative treatment the rat had gained 27 gm. in body weight. Hereafter the rat was fed the rat stock feed and gained 57 grams in weight 24 days after the treatment was started.

Rat No. 3.—Body weight 202 grams.

Symptoms as described above were observed, although they were in no way so severe as in the case of Rat No. 2 (see photographs). This rat could still walk with ease. The eyes, however, showed keratinization of the eyelids. The appetite was impaired and inactivity was evident.

After curative treatment the rat increased its bodyweight by 16 gm. in the first 10 days. In 24 days after the first treatment the rat was restored to perfect health and had gained in all 20 grams.

Rat No. 4.—Body weight 198 grams.

Symptoms as described under the "general symptoms" were observed. The eyes were severely affected, and degeneration thereof was obvious, as well as turbidity of the cornea and keratinization of the eyelids. There was a catarrhal discharge of the nasal chambers, and laboured breathing, due to sensitivity towards heat. This rat also showed deafness and took no notice of noises.

Curative treatment resulted in the rat gaining its normal vigour and health. The eyes very quickly (in a few days) became bright and open. The appetite was restored. In feeding vitaminized rat stock ration the animal gained 17 grams body weight in 12 days.

The rats in all cases (one in extremis died overnight) responded to treatment with vitamin A in the form of fish liver oil diluted with olive oil to an appropriate strength. At first the oil was given orally, a few drops at

a time. Later the feed was vitaminized with a few drops of oil, and as the appetite became normal again the balanced rat stock ration of this Institute was given.

SUMMARY.

1. Rats of 5-6 weeks of age with a vitamin A-reserve of about 1,200 I.U., when fed a vitamin A deficient ration depleted their reserves in the livers in 24 weeks time.

2. Characteristic symptoms were observed in the rats of the same ages after the 26th week when fed the same vitamin A-deficient ration; these are described in detail.

The rats when given curative treatment by dosing vitamin A responded very readily and regained normal vitality and appetite. Body weight increases were recorded.

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I herewith record my thanks to Mr. T. Meyer for the photographs.

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GRAPH I.

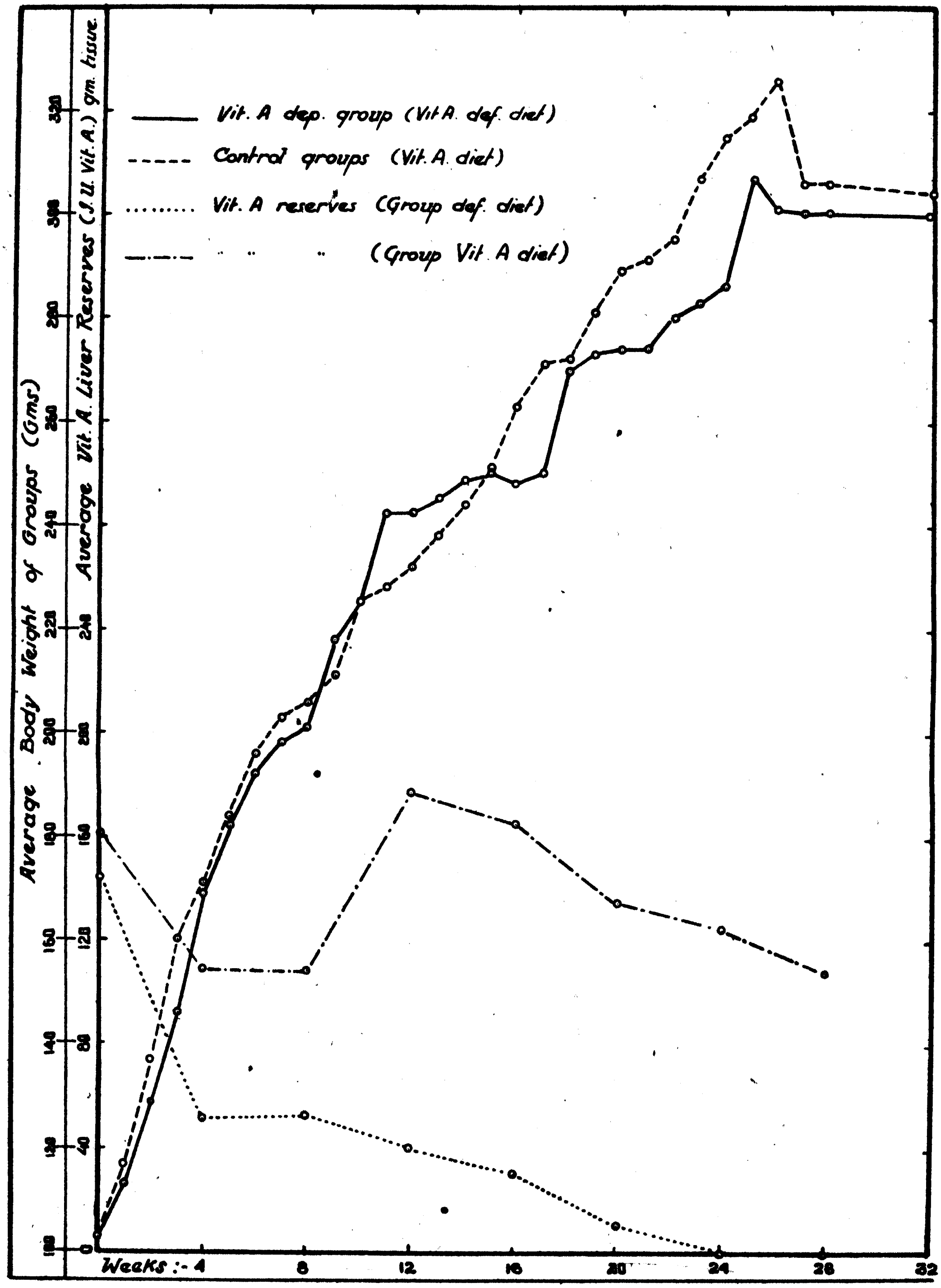
Vit. A dep. group (Vit A. def. diet)
Control groups (Vit. A. diet)
Vit. A reserves (Group def. diet)
" " " (Group Vit. A diet)
Average Body Weight of Groups (Gms)
Average Vit. A. Liver Reserves (I.U. Vit. A.) gm. tissue.
Weeks :- 4
8
12
16
20
24
28
32

Fig. 1.

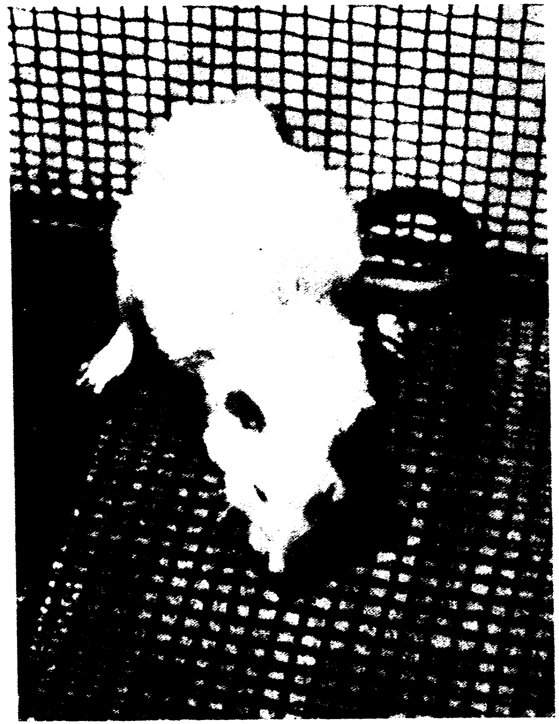

Fig. 2.

Fig. 1.—Control Group. Weight 296 gms. Age 8½ months.

Fig. 2.—Rat No. 2. Vitamin A deficient Group. Weight 180 gms. Age 8½ months.

Fig. 3.

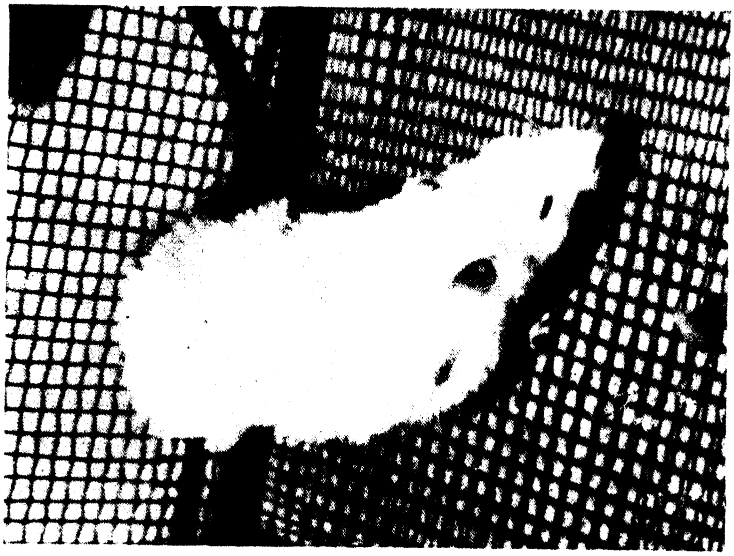

Fig. 4.

Fig. 3.—Rat No. 3. Vitamin A deficient Group. Weight 202 gms. Age $8\frac{1}{2}$ months.

Fig. 4.—Rat No. 4. Vitamin A deficient Group. Weight 198 gms. Age $8\frac{1}{2}$ months.

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Feeding Vitaminized Peanut Butter to Rats to Confirm Chemical Assays of Vitamin A.

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CHEMICAL assays for Vitamin A in products of animal origin, have been utilized by many workers since the colour tests of Carr-Price (1926) were standardised and the extraction and evaluation of the vitamin was advocated by Moore (1930). Since then the standardised chemical method has been found to be of practical value giving valid results, which may not actually be precise, but time-saving, simple and cheap, compared with the laborious, expensive, but more accurate biological methods.

Evidence has been presented by various workers, Rosenheim and Webster (1927), Wokes and Willmott (1927), Ahmad and Drummond (1930), Moore (1931), to show the practical value of the colour reaction as a means to assay Vitamin A in liver oils, etc.

The purpose of the present work was to obtain confirmation for results obtained on vitaminized peanut butter by the application of the chemical method. Repeated assays, on samples of this butter to which vitamin A had been added in the form of fish liver oils in the processing, gave an average value of 69 International (I.U.) per gram peanut butter. In employing the biological tests, the doses given were calculated from this value, which was obtained by chemical means. Thus small doses of 30 mgm. then represent 2·1 I.U. of vitamin A per dose.

EXPERIMENTAL.

Series No. 1.

Young rats (males and females) at weaning stage and three weeks old, were used. The rats were of nearly the same age and had low reserves of vitamin A in their livers. This was established by slaughtering a few rats and an average value of about 10 I.U. of vitamin A for the whole liver was found.

Precautions were taken to ensure this low reserve by feeding the mother rats the deficient diet after the second week of lactation; the young sucklings could thus not enhance their reserves by nibbling the feed or feeding thereon. It is very essential that the young rats should not have too high a reserve of vitamin A in the livers, as pointed out by various workers (e.g., Davies and Moore, 1937), since the depletion period would thereby be unnecessarily prolonged, and may be extended from weeks to months. That the young rats do build up a substantial reserve in their livers when feeding on a diet rich in vitamin A, was indicated by these authors (1937).

Body weights were recorded at short intervals and the increase in body weight was used as a criterion of vitamin A deficiency. The percentage composition of the vitamin A-deficient ration is given below:—

Modified Bacharach (1931) Ration.	Casein (ether and alcohol extracted) ...	18·5 gm.
	Dextrinised Starch	50·0 gm.
	Lard	12·3 gm.
	Yeast	10·0 gm.
	Canesugar	4·08 gm.
	Salts [Kellermann (1939)—Modified Steenbock (No. 40]	5·12 gm.

Fresh water and feed were supplied daily *ad lib.* General care was exercised, such as ventilation and temperature control of the rathouse, hygiene, exercising the rats for 10 minutes every day in direct sunlight, etc.

After a preparatory period of depletion of vitamin A-reserves, practically none, if any, vitamin A was left in reserve, after 43 days, when the curative period was started. A few rats were slaughtered at this stage and liver assays proved that the vitamin A in the livers was completely exhausted. The biological tests were done according to the technique described by Coward (1938). For the curative period the rats (with depleted livers) were grouped by using the Random Numbers of Fisher—3 males and 2 females in each of three groups, according to body weight.

This experiment served as a preliminary test to enable the author to ascertain whether the doses given in the biological feeding tests ensured normal growth or not. The vitaminized peanut butter was, therefore, fed in small doses. Such preliminary feeding tests to obtain the minimum dose necessary to ensure normal growth, were advocated by Hume and Smith (1928). For example, 0·5 gm. butter daily would not suffice for normal growth when fed to rats, but caused premature flattening in the growth curve, whereas 1·0 gm. butter daily gave a normal growth curve to maturity (Hume and Smith, 1928).

According to Goss and Guilbert (1939) the minimum daily requirement of rats for normal growth, etc., was 18-22 I.U. of vitamin A per Kgm. body weight. Calculated on this basis, a male rat (178 gm.) needs 3·2-3·9 I.U. daily and a female (138 gm.) needs 2·5-2·75 I.U. daily.

Group A (3 *males and* 2 *females*).

The deficient ration was fed *ad lib*. Fish Liver oil (diluted down with olive oil to the appropriate strength) was administered daily, at the rate of 2·1 I.U. of vitamin A per rat daily.

Group B (3 *males and* 2 *females*).

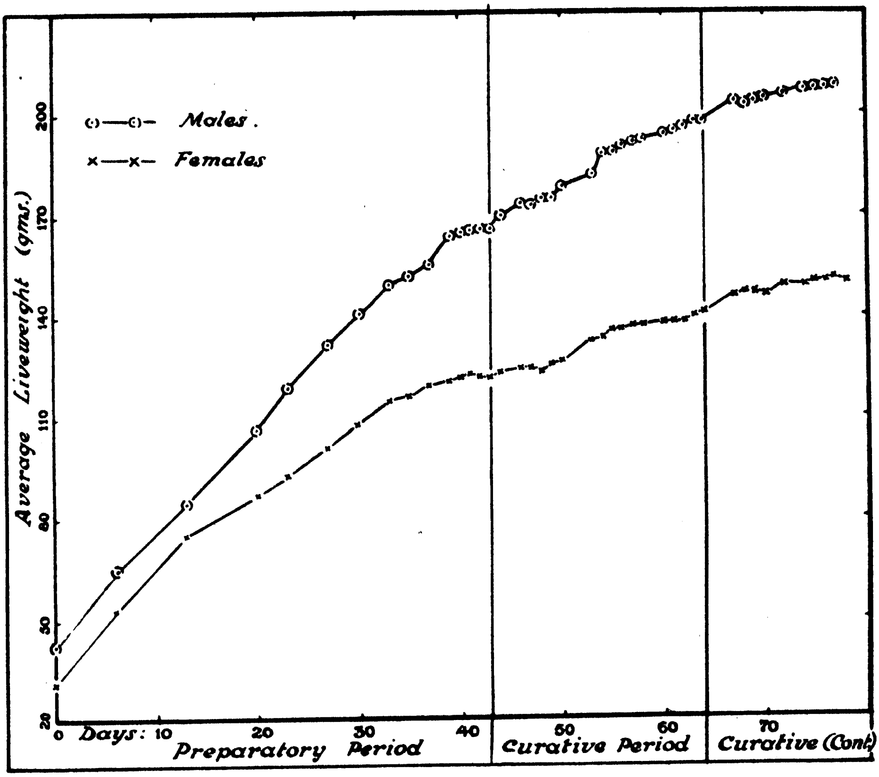
The deficient ration was fed *ad lib*. Vitaminized peanut butter (the butter itself lacked vitamin A) was fed at the rate of 2·1 I.U. of vitamin A per rat daily. In processing, the same fish liver oil was used in the peanut butter, as for the sample dosed in Group A. Bi-weekly dosages of peanut butter were employed as advocated by Coward (1934).

Group C (3 *males and* 2 *females*).

The deficient ration was fed *ad lib*. The vitaminized peanut butter was fed in portions equivalent to 4·2 I.U. vitamin A per rat daily. Bi-weekly dosages were given. The feeding of the peanut butter was as follows: The

doses were weighed out on glass slides in flat bottom glass basins (Petrie Basins), so that the rat could not take the slide in its mouth. In this way, by close observation, one could make sure that the animal had consumed all. By moistening the butter with a drop or two of olive oil, the rats gave no trouble at all and readily devoured the small quantity of butter given them. Weighings were carried out, at first weekly, then bi-weekly, and in the last stages of the preparatory period, when the weights became constant, daily. In the Curative Period the weighings were carried out every second day or daily.

GROUP A (Series No. 1).



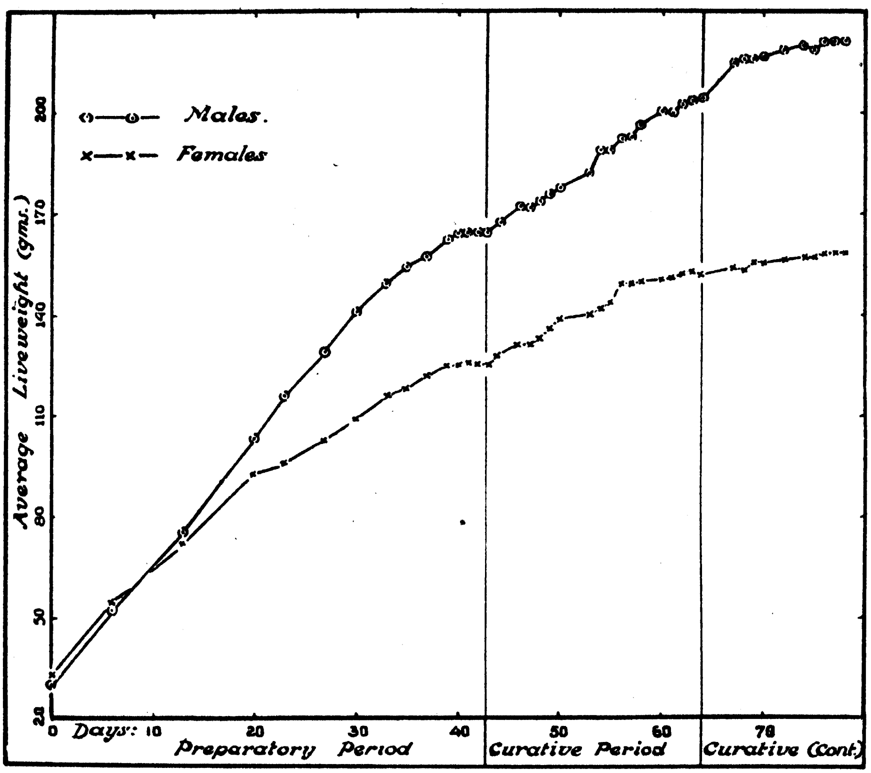
Series No. 2.

In a second series of tests where rats were given vitamin A, improvements were made on Series No. 1.

Here equal numbers of males and females were used in each group, which was increased by one animal. In the three groups there were thus six animals each. Group D was the control (where no vitamin A was given). Group E was given vitaminized peanut butter at the rate of 60 mgm. (=4·2 I.U. Vit. A) per rat daily. Group F was given 120 mgm. (=8·4 I.U. Vit. A) per rat daily.

It was obvious from the preliminary tests that waiting overlong for the rats to become steady in weight in the preparatory period, before initiating the curative period, resulted in a slow response of some rats to curative treatment. There was a lag in growth response and the rats concerned could not compete with their group mates. As a result such a group, where this setback occurred, could not compete with another group where no such setback was found. The females were most liable to such behaviour. In the 2nd series, the rats were carefully observed and when about 60 per cent. of animals became steady in weight for about three days the curative period was begun.

GROUP B (Series No. 1).



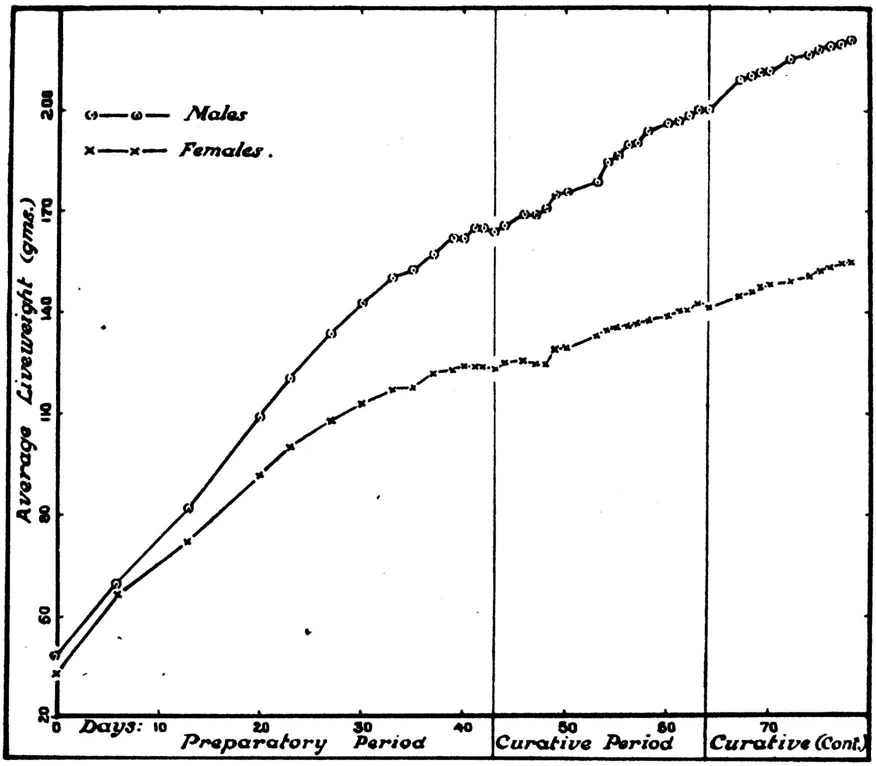
From the growth curves (for 5 weeks dosing) in the previous series, it was evident that doses of 2·1 I.U. vitamin A per rat daily (for fish oil as well as peanut butter) were too small to yield normal growth curves. Hence doses of double strength, viz., 4·2 I.U. and also 8·4 I.U. were taken.

Discussion.

Positive growth results were obtained when vitaminized peanut butter had been fed to rats previously depleted of their vitamin A reserves. The depletion on preparatory periods lasted 43 days in the case of Series No. 1, and 52 days in Series No. 2. The curative period was 21 days and there was response in every case where the peanut butter had been dosed. By

prolonging the curative periods for 35 days without a break, it could be established whether normal growth was taking place. In Series No. 1 the rats of Groups A and B grew on an average sub-normally, as compared with Group C notwithstanding the fact that a few rats of this group lagged behind in body weight returns in the initial stages of curing. Group B appeared to be best favoured in this respect and there was a quick growth response to vitamin A. In Series No. 2, positive growth responses were found in both Groups E and F, where vitamin A had been given, but Group D (controls)

GROUP C (Series No. 1).



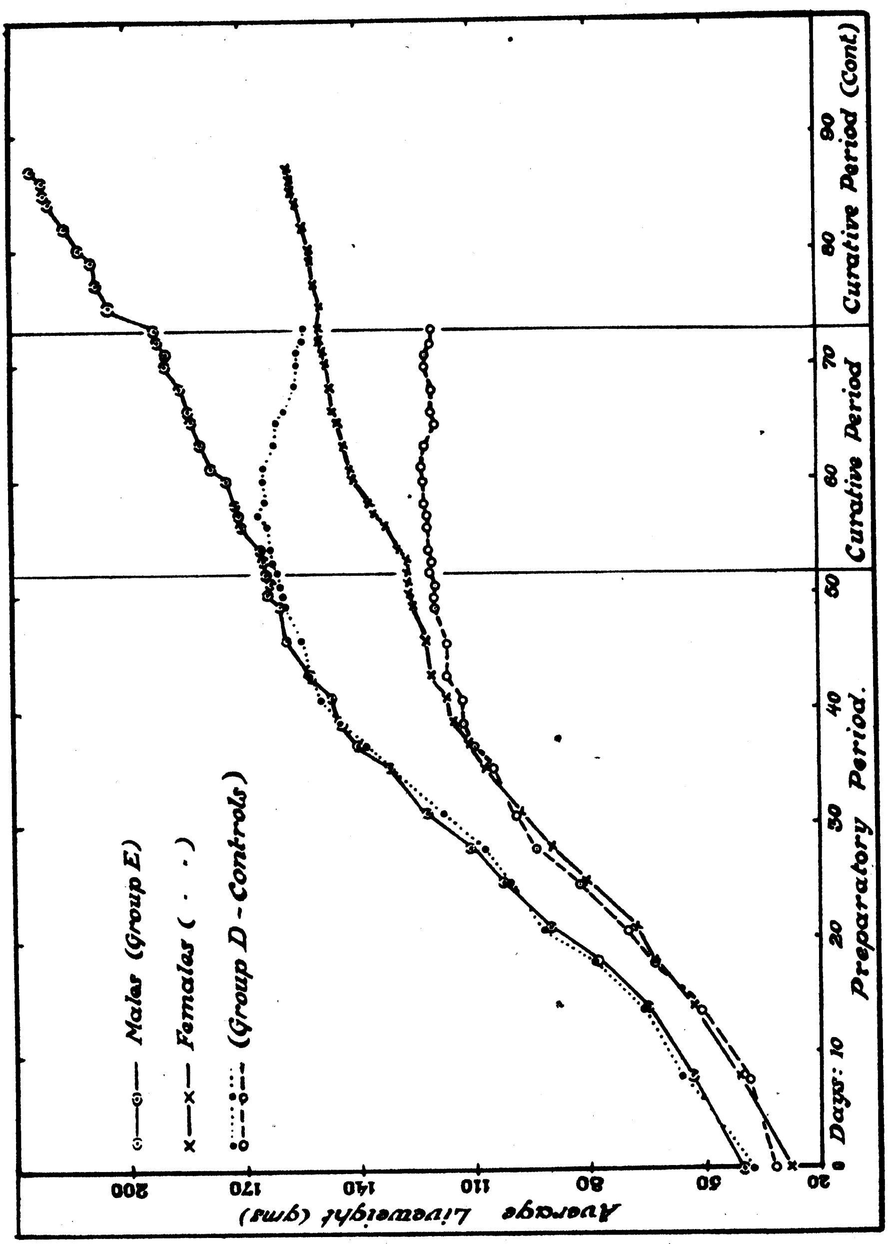
showed in most cases loss in body weight or a tendency to cease growing or to grow irregularly with small gains for the period. In these young rats of less than 3 months of age, typical symptoms of vitamin A deficiency were observed which are here described.

Group D.

Rat No. 1. (*Male.*)

Remarkably constant body weight, since the termination of depletion period was registered. Four days after this date (23.10.41) the first obvious symptom was seen, viz., light-sensitivity of the left eye (partly closed eyelids). At the end of eight days both eyes were affected; a discharge of the right eye was apparent; deterioration of the eye gland was taking place.

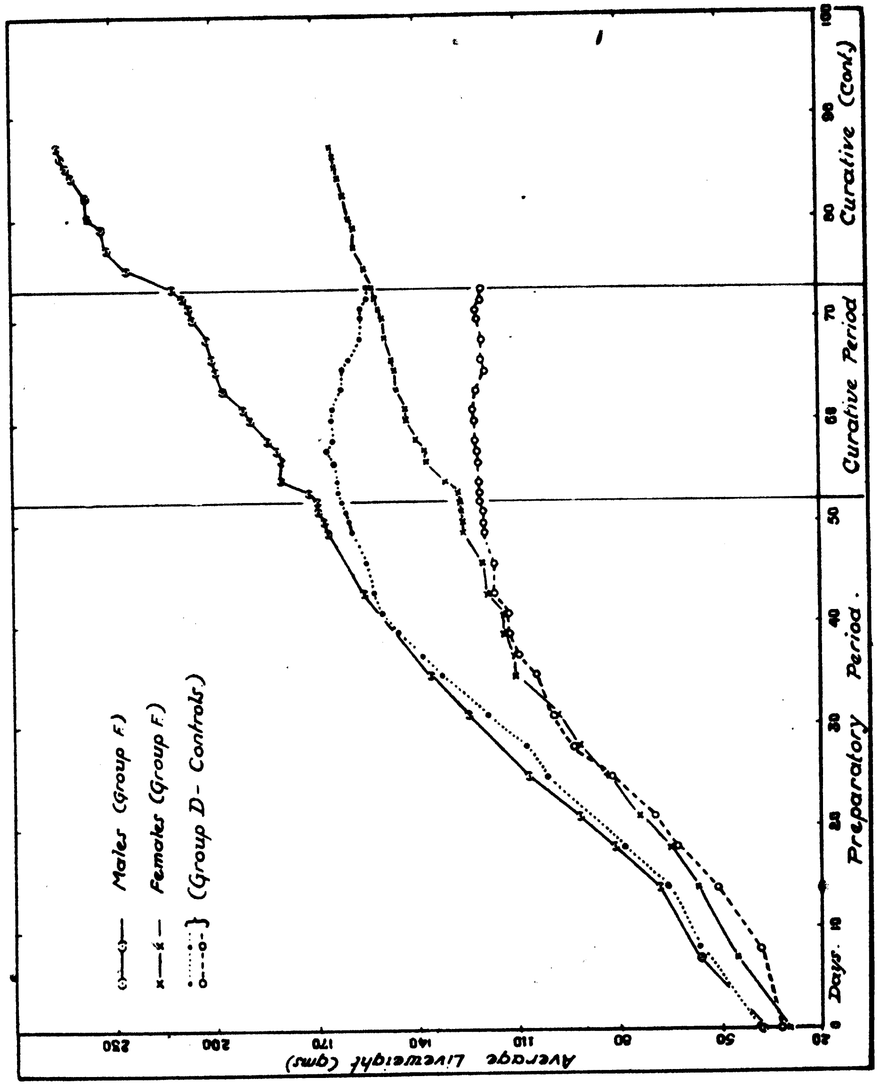
The next day, the rat lost its appetite which did not return before vitamin A curative dosing was practised at a later stage. On the 13th day the rat was listless, and obviously ill. The eyes were dull and keratinization of the eyelids was observed. On the 15th day, difficulty to walk normally, due partly to inactivity and partial lameness (paresis) was seen. On examination of the mucous membrane of the lower jaw, tainted gums of incisors



GROUP E (Series No. 2).

were observed. A catarrhal discharge of the nose, sticky and brown in colour, appeared on the 19th day. As the rat was very ill and would have died since it was wasting away quickly, curative treatment by dosing diluted fish liver oil, was practised. The rat revived, appetite was restored, body weight increased remarkably, and the general health returned. After a short period of four days, the rat increased its body weight by 10 grams due largely to an enhanced appetite.

GROUP F (Series No. 2).



Rat No. 2. (Male.)

Body weight showed an ultimate drop.

After 10 days depletion: Right eye affected.

After 13 days depletion: Light-sensitivity.

After 15 days depletion: Animal crouched and became listless.

After 17 days depletion: The animal showed paresis of the hind legs.

After 18 days depletion: Appetite impaired. There was a catarrhal discharge of left nasal chamber. Gums were bloody on lower jaw.

After 19 days depletion: Increased nasal discharge.

After 20 days depletion: Rat apparently deaf.

Hereafter the animal was given curative treatment and restored to health and vigour.

Rat No. 3. (*Male.*)

Ultimate drop in body weight.

After 4 days depletion: Right eye affected—light sensitive.

After 6 days depletion: Right eye, discharge from gland.

After 9 days depletion: Left eye almost completely closed. Animal twitches, nervous and restless.

After 17 days depletion: Left eye purulent; breathing laboured; rat walks with difficulty dragging the body along and goes down on haunches, in a sprawling attitude, unable to support its body on hind legs. Walks with a limp.

After 19 days depletion: Inco-ordination of limbs in walking and sways from side to side. Listless and very sensitive to the touch but otherwise very tame. There was a discharge from the nose of brownish fluid.

After 20 days depletion: Rat appeared very ill.

On the 21st day the rat was killed for vitamin A assay of the liver (negative result). At post-mortem there was oedema of the lungs.

Rat No. 4. (*Female.*)

Remarkably steady body weight which dropped after one week.

After 8 days depletion: Left eye was sensitive to light.

After 14 days depletion: Right eye shows discharge from the gland.

After 16 days depletion: Animal walks with difficulty, drags body along.

After 18 days depletion: Severely affected gums and red patches on mucous membrane of lower mouth parts.

After 19 days depletion: Sniffles; husky breathing, probably affected lungs.

After 21 days depletion: Gums generally affected.

Rat killed for liver test for vitamin A (negative result).

Post-mortem revealed nothing unusual in the lungs.

Rat No. 5. (*Female.*)

Rather irregular, but small gain in body weight.

After 13 days depletion: Right eye light sensitive.

After 16 days depletion: Twitching of muscles. Walks with difficulty.

After 18 days depletion: Appetite had been fairly good all along but now apparently lost. Discharge, though scant, of the nose.

After the 21st day the animal was given vitamin A (oil) orally. A considerable gain in body weight was observed, 11 grams in 4 days, due to enhanced and restored appetite. The rat was quick in response to dosing and showed brightness of the eyes in comparison with previous dullness.

Rat No. 6. (*Female.*)

Showed about 7 grams increase in body weight for the three weeks. No obvious symptoms, except for a slight discharge of the right eye on the 20th day.

The rat was hereafter dosed with vitamin A and restored to vigorous growth . In 4 days of vitamin A administration the animal gained 11 grams in body weight.

Liver Assays.

In order to ascertain what reserves (if any) of vitamin A were stored in the livers during the curative periods, at the various stoges, liver assays were carried out as follows:—

In both Series No. 1 and No. 2:

(*a*) 2 rats (male and female) were slaughtered at weaning (three weeks after birth).

(*b*) 2 rats (male and female) were slaughtered at the end of preparatory periods.

(*c*) 2 rats in each group (A-C and E-F) at the end of 21 days of curative period.

(*d*) the residual rats of each group at the end of 5th week of vitamin A supplementation (see Tables 3 and 6 in the appendix).

Small reserves of vitamin A were found at the initial stages. Storage had taken place during the gestation period and lactation period of the mothers. Due to controlled feeding of vitamin A to the mother, the reserve of vitamin A in the young sucklings was kept low (about 10 I.U.).

At the end of preparatory periods no vitamin A could be found on testing the liver reserves proving that in all the rats the livers had probably been thoroughly depleted in the short time of 43-52 days.

At the end of the curative period of 21 days no vitamin A had been stored in the liver in Groups A, B and C in Series No. 1. Hence 2-4 I.U. of vitamin A per rat daily were, therefore, enough to prevent symptoms and grant a certain level of growth but allowed no reserves to be built up in this time. In Series No. 2, however, a small reserve was stored in the livers of the rats in Group F at the end of 21 days but none in the rats of Group E. In other words, where 4·2 I.U. vitamin A per rat daily was insufficent to allow a reserve to be stored in the livers after 21 days, 8·4 I.U. vitamin A per rat daily allowed a small quantity in reserve. Again, after the expiration of 5 weeks of the curative period, the rats slaughtered (Groups E and F) showed no storage in Group E and a small storage in Group F. The female rat of the last-named group had stored as much as 3·6 I.U. vitamin A in the whole liver. The other members of this group had only stored a small reserve of about 0·6 I.U. due largely to the requirements being larger and thus the demand was bigger than for the female, especially where the male

registered a rate of growth of 85 gm. in 5 weeks. It is to be noted that the rats of the control group, when cured after the expiration of 21 days during which time typical symptoms were observed, very quickly responded to treatment and gained rapidly in weight thereafter.

In conclusion, the daily doses of 4·2 I.U. and 8·4 I.U. vitamin A calculated from the value 1 gm. = 69 I.Us. based on chemical analyses, were found by biological tests to be adequate to give positive growth responses, as can be gathered from the graphs. Chemical analyses are thus valuable indications of the vitamin A value of foods.

Summary.

Positive results were obtained when vitaminized peanut butter was fed in small doses of 4·2 and 8·4 I.U's. of vitamin A per rat daily. Body weight increases served as criteria. For the same peanut butter, on chmical tests an average value of 69 I.U's. of vitamin A per gram was found at the initial stages of the biological tests.

The doses were calculated on the chemical findings and on this basis doses of 2·1 I.U's were too small, whilst doses of 4·2 I.U. were adequate; and doses of 8·4 also allowed a small storage in the liver within 21 days of feeding. The above findings support the views of Goss and Guilbert (1939) who advocated a minimum level of vitamin daily of 18-22 I.U. per Kgm. weight in rats, which, calculated on the average weight of the rats in this experiment is equivalent to 2·5-3·9 I.U. vitamin A per dose per rat daily.

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TABLE 1.

Body Weight of Rats (Gm.).

Preparatory Period.—Series No. 1 (Duration 43 Days).

Rat No.	Sex.	15/8/41.	20/8/41.	27/8/41.	3/9/41.	6/9/41.	10/9/41.	13/9/41.	16/9/41.	18/9/41.	20/9/41.	22/9/41.	23/9/41.	24/9/41.	25/9/41.	26/9/41.
1	Male......	64	83	99	119	133	145	149	157	160	164	168	170	170	170	170
2	Male......	30	53	84	110	124	136	148	159	165	171	176	177	178	179	180
3	Male......	32	58	73	88	99	116	126	135	139	142	147	147	148	148	148
4	Female....	35	56	79	88	94	102	111	118	119	123	123	124	126	125	125
5	Female....	28	51	71	86	93	100	105	112	113	117	119	120	120	119	120
Average.	Male......	42	65	85	106	119	132	141	150	155	159	164	165	166	166	166
	Female....	31	53	75	87	93	101	108	115	116	120	121	122	123	122	122
6	Male......	29	59	85	108	118	132	142	145	149	151	155	155	156	156	155
7	Male......	29	50	69	97	150	118	129	139	144	147	152	154	156	156	156
8	Male......	32	48	70	104	124	137	152	164	169	173	178	182	181	181	182
9	Female....	34	60	80	100	101	104	110	118	119	123	126	126	127	125	125
10	Female....	32	50	64	86	94	102	109	114	117	121	124	124	127	125	126
Average.	Male......	30	52	75	103	116	129	141	149	154	157	162	164	164	164	164
	Female....	33	55	72	93	97	103	109	116	118	122	125	125	126	125	125
11	Male......	51	71	95	123	137	153	161	168	169	172	176	177	179	179	178
12	Female....	34	56	71	98	110	124	134	140	146	151	156	155	158	158	157
13	Male......	30	54	81	107	116	126	134	144	145	151	153	153	157	157	157
14	Female....	34	56	65	90	98	108	114	120	122	125	127	128	128	127	126
15	Female....	33	60	77	93	101	108	112	115	115	119	120	120	121	120	120
Average.	Male......	38	60	82	109	121	134	143	151	153	158	162	162	165	165	164
	Female....	33	58	72	92	100	108	113	117	118	122	123	124	124	124	123

TABLE 2.

Body Weight of Rats (Gm.).

Curative Period.—Series No. 1 (Duration 21 Days).

Group	Rat No.	Sex.	26/9/41.	27/9/41.	29/9/41.	30/9/41.	1/10/41.	2/10/41.	3/10/41.	6/10/41.	7/10/41.	8/10/41.	9/10/41.	10/10/41.	11/10/41.	13/10/41.	14/10/41.	15/10/41.	16/10/41.	17/10/41.	Gains.*	Remarks.
GROUP A. (2 I.U. Vit. A. per rat daily).	1	Male......	170	174	179	179	181	181	183	185	190	191	192	194	193	194	195	196	196	196	+26	Curative period continued on to 5 weeks.
	2	Male......	180	183	186	185	187	187	192	194	198	200	202	203	204	206	207	208	210	213	+30	Curative period continued on to 5 weeks.
	3	Male......	148	152	156	155	158	161	163	169	177	177	179	180	182	184	185	187	189	192	+41	Killed for Liver Vit. A. Assays.
	4	Female....	125	125	129	129	124†	127	130	134	136	137	139	141	140	140	141	140	144	146	+18	Killed for Liver Vit. A. Assays.
	5	Female....	120	124	122†	121	123	124	126	128	130	130	133	133	134	136	135	137	137	137	+16	Curative period continued on to 5 weeks.
	Average.	Male......	166	170	174	173	175	175	179	182	189	189	191	192	193	194	195	196	198	198	+32	—
		Female....	122	124	125	125	124	126	128	133	134	136	136	137	137	138	138	138	140	141	+17	—
GROUP B. (2 I.U. Vit. A. per rat daily).	6	Male......	155	158	157	152†	155	156	158	161	168	168	170	172	176	180	180	181	183	186	+28	Killed for Liver Vit. A. Assays.
	7	Male......	156	160	166	168	171	173	175	181	187	188	191	192	195	198	198	201	202	201	+45	Curative period continued on to 5 weeks.
	8	Male......	182	184	192	192	195	198	200	204	212	212	215	216	217	223	223	225	226	227	+44	Curative period continued on to 5 weeks.
	9	Female....	125	129	131	130	132	134	137	138	139	145	149	148	150	150	150	153	150	150	+27	Killed for Liver Vit. A. Assays.
	10	Female....	126	128	131	132	134	139	140	143	145	144	149	149	150	151	152	152	153	152	+26	Curative period continued on to 5 weeks.
	Average.	Male......	164	167	172	171	174	176	178	182	189	189	192	193	196	200	200	203	204	205	+39	—
		Female....	125	128	131	131	133	136	139	140	142	144	149	149	150	150	151	152	153	152	+26	—
GROUP C. (4 I.U. Vit. A. per rat daily).	11	Male......	178	178	181	183	185	190	191	197	20[illegible]	202	206	207	210	212	211	214	217	215	+37	—
	12	Male......	157	161	163	160†	160	163	164	167	173	177	180	180	184	184	185	187	189	190	+32	—
	13	Male......	157	158	164	165	169	171	172	174	180	181	184	187	189	193	193	194	196	196	+38	—
	14	Female....	126	129	133	130†	130	135	133†	136	135†	135	136	137	138	138	139	140	143	143	+16	—
	15	Female....	120	123	120†	120	121	126	127	130	136	136	137	137	139	140	140	141	143	142	+22	—
	Average.	Male......	164	166	169	169	171	175	176	179	185	187	190	191	194	196	196	198	200	200	+36	—
		Female....	123	126	126	125	125	130	130	133	135	136	136	137	138	139	140	141	143	142	+19	—

* Gains calculated on the average weights for last 3 days.

† Rats suffered temporary setback.

TABLE 2 (*a*).

Body Weight of Rats (Gm.).

Continuation of Curative Period (to 35th day)—Series No. 1.

	Rat No.	Sex.	20/10/41.	21/10/41.	22/10/41.	23/10/41.	25/10/41.	27/10/41.	28/10/41.	29/10/41.	30/10/41.	31/10/41.	Remarks.	
Group A.	1	Male......	196	197	197	197	200	201	200	201	202	204	Total gain (35 days) = + 34 gm.	
	2	Male......	212	209	211	212	212	213	214	214	215	214	Total gain (35 days) = + 34 gm.	Rat killed for Liver test.
	4	Female....	146	147	147	146	149	149	150	150	151	150	Total gain (35 days) = + 25 gm.	Rat killed for Liver test.
Group B.	7	Male......	203	203	202	204	205	206	204	205	205	204	Total gain (35 days) = + 48 gm.	Rat killed for Liver test.
	8	Male......	227	228	229	230	233	235	234	236	236	237	Total gain (35 days) = + 55 gm.	
	10	Female....	154	153	155	155	156	156	157	160	160	160	Total gain (35 days) = + 34 gm.	Rat killed for Liver test.
Group C.	11	Male......	218	218	219	220	222	222	224	226	226	228	Total gain (35 days) = + 50 gm.	Rat killed for Liver test.
	13	Male......	200	201	203	203	208	209	212	212	213	216	Total gain (35 days) = + 59 gm.	Rat killed for Liver test.
	14	Female....	145	146	148	149	149	151	153	154	155	155	Total gain (35 days) = + 29 gm.	

TABLE 3.

Liver Assays for Vitamin A Reserves. Rats in Series No. 1.

	Rat No.	Sex.	Preparatory Period.								Curative Period.							
			Initial Stage.				End Stage.				After 21 days.				After 35 days.			
			Weight.	Liver. Weight.	Vit. A. I.U.	Storage I.U.	Weight.	Liver. Weight.	Vit. A. I.U.	Storage I.U.	Weight.	Liver. Weight.	Vit. A. I.U.	Storage I.U.	Weight.	Liver. Weight.	Vit. A. I.U.	Storage I.U.
			Gms.	Gms.			Gms.	Gms.			Gms.	Gms.			Gms.	Gms.		
	—	Male	40	2·1	12·0	12·0	—	—	—	—	—	—	—	—	—	—	—	—
	—	Female	27	1·4	13·4	13·4	—	—	—	—	—	—	—	—	—	—	—	—
	16	Male	—	—	—	—	159	8·1	0	0	—	—	—	—	—	—	—	—
	17	Female	—	—	—	—	125	6·7	0	0	—	—	—	—	—	—	—	—
Group A.	3	Male	—	—	—	—	—	—	—	—	189	9·8	0	0	—	—	—	—
	5	Female	—	—	—	—	—	—	—	—	136	6·3	0	0	—	—	—	—
Group B.	6	Male	—	—	—	—	—	—	—	—	183	8·6	0	0	—	—	—	—
	9	Female	—	—	—	—	—	—	—	—	152	7·5	0	0	—	—	—	—
Group C.	12	Male	—	—	—	—	—	—	—	—	189	10·3	0	0	—	—	—	—
	15	Female	—	—	—	—	—	—	—	—	142	5·8	0	0	—	—	—	—
Group A.	2	Male	—	—	—	—	—	—	—	—	—	—	—	—	214	8·0	0	0
	4	Female	—	—	—	—	—	—	—	—	—	—	—	—	150	6·5	0	0
Group B.	7	Male	—	—	—	—	—	—	—	—	—	—	—	—	205	9·7	0	0
	10	Female	—	—	—	—	—	—	—	—	—	—	—	—	159	7·0	0	0
Group C.	11	Male	—	—	—	—	—	—	—	—	—	—	—	—	227	8·0	0	0
	14	Female	—	—	—	—	—	—	—	—	—	—	—	—	155	6·0	0	0

TABLE 4.

Body Weight of Rats (Gm.).

Preparatory Period.—Series No. 2 (Duration 52 Days).

Rat No.	Sex.	Date: 1941.																	
		2/9	10/9	16/9	20/9	23/9	27/9	30/9	3/10	7/10	9/10	11/10	13/10	15/10	17/10	20/10	21/10	22/10	23/10
1	Male	44	66	75	87	103	115	117	127	141	147	155	159	162	161	168	169	170	171
2	Male	42	55	65	77	92	96	104	114	128	133	140	142	148	153	156	158	158	160
3	Male	27	46	58	70	81	93	101	112	128	135	139	141	147	149	152	152	153	154
4	Female	31	41	54	66	73	85	92	100	106	112	115	117	120	121	124	125	125	125
5	Female	27	36	49	60	63	81	87	95	100	104	107	109	112	113	115	115	117	117
6	Female	28	36	46	59	70	80	90	100	106	111	114	114	119	116	119	117	117	118
Average	Male	38	56	66	78	92	101	107	118	132	138	145	147	152	154	159	160	160	161
	Female	32	38	50	62	69	82	90	98	104	109	112	113	117	116	119	119	120	120
7	Male	44	64	75	90	106	125	131	142	155	158	160	162	169	172	175	178	178	178
8	Male	37	52	65	77	90	98	105	120	138	142	150	153	159	159	165	169	168	168
9	Male	30	45	56	66	75	85	96	104	112	120	126	126	133	136	141	142	141	142
10	Female	30	47	48	61	65	81	92	100	107	111	116	118	123	124	129	131	129	129
11	Female	27	43	53	61	68	77	86	92	103	108	111	114	119	120	124	126	127	127
12	Female	28	44	58	67	70	85	92	101	110	113	117	118	120	123	124	123	125	125
Average	Male	37	54	65	78	90	103	111	122	135	140	145	147	153	159	160	163	162	163
	Female	28	41	53	63	68	81	90	98	107	111	115	117	121	122	126	127	127	127
13	Male	50	70	86	105	115	125	—	145	153	—	—	—	178	—	198	199	203	203
14	Male	30	47	58	68	79	96	104	113	126	129	134	140	145	145	152	153	155	155
15	Male	31	52	61	69	82	96	105	115	129	134	140	144	146	149	149	150	149	150
16	Female	39	54	63	69	74	88	95	102	109	112	119	119	123	126	130	132	133	134
17	Female	26	43	58	66	78	80	92	100	118	112	115	118	120	120	128	128	128	128
18	Female	27	38	50	60	69	84	89	96	106	110	112	115	115	117	119	117	120	120
Average	Male	37	56	68	81	92	106	—	124	136	—	—	—	156	—	166	167	169	169
	Female	31	45	57	65	74	84	92	99	111	111	115	114	119	121	126	126	127	127

TABLE 5.

Body Weight of Rats (Gm.).

Curative Period.—Series No. 2 (Duration 21 Days).

Group	Rat No.	Sex.	23/10.	24/10.	25/10.	27/10.	28/10.	29/10.	31/10.	1/11.	3/11.	5/11.	6/11.	8/11.	10/11.	11/11.	12/11.	13/11.	Gains.	Remarks.
			DATE: 1941.																	
CONTROL GROUP D.	1	Male	171	172	172	172	172	171	171	170	168	168	165	163	164	164	163	162	− 8	Restored to health, 14/11/41.
	2	Male	160	159	160	162	161	161	162	162	162	163	162	161	161	160	159	160	− 1	Restored to health, 14/11/41.
	3	Male	154	154	155	156	156	157	156	156	153	151	149	145	143	143	142	140	−12	Killed for Liver Vit. A. Assays, 13/11/41.
	4	Female	125	125	126	125	126	126	124	125	124	124	122	122	112	112	112	113	−13	Killed for Liver Vit. A. Assays, 13/11/41.
	5	Female	117	116	118	118	119	119	120	120	120	115	116	117	119	119	120	119	+ 2	Restored to health, 14/11/41.
	6	Female	118	117	117	119	119	119	121	122	122	123	122	121	125	125	123	122	+ 5	Restored to health, 14/11/41.
	Average	Male	162	162	162	163	166	163	163	163	161	161	159	156	156	156	154	154	− 7	
		Female	119	119	120	121	121	121	122	122	122	119	120	120	122	122	118	118	− 2	
GROUP E.—(4·2 *I.U. Vit. A. per rat daily*).	7	Male	178	179	181	186	187	187	192	194	197	202	204	205	210	207	210	212	+32	Curative tests continued on to 5 weeks.
	8	Male	168	170	171	176	177	179	183	185	190	191	191	192	193	192	194	195	+26	Curative test continued on to 5 weeks.
	9	Male	142	142	144	147	150	150	154	154	156	157	158	162	166	167	169	168	+26	Killed for Liver Vit. A. Assays, 13/11/41.
	10	Female	129	129	131	134	136	138	140	141	142	143	144	144	146	145	145	146	+16	Curative test continued on to 5 weeks.
	11	Female	127	127	130	132	135	137	140	141	142	144	145	145	147	148	147	148	+21	Killed for Liver Vit. A. Assays, 13/11/41.
	12	Female	125	125	129	133	134	136	142	144	146	149	149	149	151	151	151	152	+26	Curative test continued on to 5 weeks.
	Average	Male	163	164	165	170	171	172	176	178	181	183	184	186	190	189	191	192	+28	
		Female	127	127	130	133	135	137	141	142	143	145	146	146	148	148	149	149	+22	
(GROUP F.—(8·4 *I.U. Vit. A. per rat daily*).	13	Male	203	207	209	211	214	218	224	229	236	239	241	2[illegible]7	251	252	255	259	+53	Curative period continued on to 5 weeks.
	14	Male	155	157	159	166	167	170	173	175	177	179	180	182	184	185	187	187	+32	Curative period continued on to 5 weeks.
	15	Male	150	152	155	162	163	164	169	173	177	179	179	183	184	184	186	187	+36	Killed for Liver Vit. A. Assays, 13/11/41.
	16	Female	134	133	136	139	140	144	145	145	148	148	148	150	150	152	154	153	+19	Killed for Liver Vit. A. Assays, 13/11/41.
	17	Female	128	130	133	137	138	137	141	142	143	147	147	147	148	148	149	153	+23	Curative period continued on to 5 weeks.
	18	Female	120	122	127	135	136	139	142	143	146	147	149	149	151	513	153	153	+23	Curative period continued on to 5 weeks.
	Average	Male	169	172	181	180	181	184	189	192	197	199	200	204	206	207	209	212	+40	
		Female	127	128	132	137	138	140	143	144	146	147	148	149	150	151	152	153	+22	

TABLE 5 (*a*).

Body Weight of Rats (Gm.).

Continuation of Curative Period (to 35th day).—Series No. 2.

	Rat No.	Sex.	Date : 1941.									Remarks.
			15/11	17/11	19/11	20/11	22/11	24/11	25/11	26/11	27/11	
Control Group D.	1	Male...	165	173	168	170	174	182	185	187	187	Rat cured ; increase in body weight 25 grms. (14 days).
	2	Male...	157	160	180	187	196	204	209	208	210	Rat cured ; increase in body weight 50 gms. (14 days).
	5	Female.	124	130	135	137	141	145	147	147	150	Rat cured ; increase in body weight 31 gms. (14 days).
	6	Female.	122	134	139	143	149	158	160	159	160	Rat cured ; increase in body weight 38 gms. (14 days).
Group E.	7	Male...	213	216	217	220	223	227	228	228	231	Total gain (35 days) = ± 53 gms. Killed for Liver Vit. A. Assays, 28/11/41.
	8	Male...	197	200	202	205	209	213	215	216	218	Total gain (35 days) = ± 50 gms.
	12	Female.	152	153	155	155	155	158	159	159	160	Total gain (35 days) = ± 35 gms. Killed for Liver Vit. A. Assays, 28/11/41.
	10	Female.	146	149	149	150	153	154	155	154	155	Total gain (35 days) = ± 26 gms.
Group F.	13	Male...	262	269	271	276	279	284	285	285	289	Total gain (35 days) = ± 85 gms. Killed for Liver Vit. A. Assays, 28/11/41.
	14	Male...	190	193	195	198	198	200	203	205	207	Total gain (35 days) = ± 52 gms.
	18	Female.	158	159	159	160	164	165	166	167	169	Total gain (35 days) = ± 49 gms. Killed for Liver Vit. A. Assays, 28/11/41.
	17	Female.	152	157	157	157	159	159	160	160	161	Total gain (35 days) = ± 33 gms.

TABLE 6.

Liver Assays for Vitamin A Reserves.

Rats in Series No. 2.

	Rat No.	Sex.	Preparatory Period.								Curative Period.							
			Initial Stage.				End Stage.				After 21 days.				After 35 days.			
			Wht.	Liver Wht.	Vit. A. I.U.	Storage I.U.	Wht.	Liver Wht.	Vit. A. I.U.	Storage I.U.	Wht.	Liver Wht.	Vit. A. I.U.	Storage I.U.	Wht.	Liver Wht.	Vit. A. I.U.	Storage I.U.
				Gms.				Gms.				Gms.				Gms.		
	—	Male	50	2·3	10·8	10·8	—	—	—	—	—	—	—	—	—	—	—	—
	—	Female	48	2·1	8·4	8·4	—	—	—	—	—	—	—	—	—	—	—	—
	—	Male	—	—	—	—	160	8·0	0	0	—	—	—	—	—	—	—	—
	—	Female	—	—	—	—	125	6·5	0	0	—	—	—	—	—	—	—	—
Group D.	3	Male	—	—	—	—	—	—	—	—	142	4·4	0	0	—	—	—	—
	4	Female	—	—	—	—	—	—	—	—	112	4·1	0	0	—	—	—	—
Group E.	9	Male	—	—	—	—	—	—	—	—	168	7·6	0	0	—	—	—	—
	11	Female	—	—	—	—	—	—	—	—	148	7·4	0	0	—	—	—	—
Group F.	15	Male	—	—	—	—	—	—	—	—	186	9·0	1·2	1·2	—	—	—	—
	16	Female	—	—	—	—	—	—	—	—	153	7·5	0·6	0·6	—	—	—	—
Group E.	7	Male	—	—	—	—	—	—	—	—	—	—	—	—	231	10·8	0	0
	12	Female	—	—	—	—	—	—	—	—	—	—	—	—	160	6·7	0	0
Group F.	13	Male	—	—	—	—	—	—	—	—	—	—	—	—	289	13·1	0·6	0·6
	18	Female	—	—	—	—	—	—	—	—	—	—	—	—	169	8·6	3·6	3·6

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The Influence of Varying Maize Supplements on the Digestibility of the Cellulose in a Poor Veld Hay in Relation to the Bacterial Population of the Rumen of Sheep with a Note on the Nitrogen Metabolism.

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INTRODUCTION.

EXTENSIVE analytical evidence was produced by du Toit *et al* (1940) in studies on the natural veld that South African pastures are deficient not only in phosphate for the greater part of the year, but also in protein, more especially during winter or other times of food scarcity, such as drought. A glance at the analytical data published reveals extraordinary high values for the crude fibre content of the pastures analysed for the greater part of the year, suggesting the probability of a shortage of easily digestible, energy-producing material, particularly when stock are entirely dependent on mature, hard and fibrous grazing. This possible deficiency was investigated by du Toit, Malan and Smuts in a series of experiments with sheep, started in 1935 on the Government Farm, Nooitgedacht, in the Ermelo district situated in the Transvaal Highveld where frosts are severe during winter. A critical survey of the data produced in these experiments indicates that the supplementation of pasture with phosphate and protein during the winter months has little effect upon the condition of sheep unless the supplement contains carbohydrate as well, suggesting that poor use is being made of the energy-producing materials of the pasture under the existing conditions. Subsequently Smuts and Marais (1940) in a series of metabolism studies produced further evidence for the existence of an energy deficiency in the winter grazing of the Transvaal.

The energy-yielding part of the dry winter grazing consists almost exclusively of cellulose and other structural polysaccharides. An animal subsisting on such grazing would, therefore, be dependent upon its ability to utilise such carbohydrates for practically the whole of its energy requirements. It is consequently a matter of some interest, if not of practical importance to determine the magnitude of the cellulose utilization in winter grazing, and to ascertain to what extent, if at all, it is modified by supplementing the grazing with feeds necessary for the rectification of the known deficiencies, viz., protein, phosphate, and energy.

It is a well-known fact that the breakdown of cellulose and the hemicelluloses is accomplished not by enzymes secreted in the digestive tract but by enzymes of symbiotic micro-organisms. The quantitative relations

involved in this microbiotic decomposition of carbohydrates are, however, suspected to be subject to variation depending upon the type and number of organisms present, which in turn are under the influence of the character of the food. Thus, it has been shown that the addition of easily digestible carbohydrates such as starch, cane sugar, or glucose to the ration of cattle or sheep reduced the digestibility of the fibre. The most recent evidence for this phenomenon has been supplied by Hamilton (1942) in experiments on sheep.

The degree of the breakdown of the structural carbohydrates is also intimately associated with their chemical and physical nature. It has often been demonstrated, for example by Louw (1942), that the complex polysaccharides of mature plants are not as well digested as they are in young, growing plants. The difference is due particularly to the presence of certain encrusting substances, notably lignin, which are deposited in increasing amount in the cell with advancing age. Micro-organisms have little or no action on lignin, especially on the lignin of mature plants, with the result that the cellulose is protected from the action of the organisms by the lignin or, probably, by a lignin-hemicellulose complex [c.f. Louw (Loc. cit.)]. Since it is known that pure isolated cellulose is almost completely digestible in the paunch of the ruminant it seems justifiable to state that in the presence of a sufficient number of the cellulose-digesting bacteria the digestion of the cellulose of mature plants (for instance, winter grazing in the Transvaal) will be limited largely by the degree of winter grazing in the Transvaal) will be limited largely by the degree of lignification. That the number and type of organisms may constitute yet another limiting factor, apart from lignification, in the digestion of the cellulose may be assumed from the work recently published by Harris and Mitchell (1941). These workers found that the addition of 5·0 grams urea to a basal ration containing only 0·136 per cent. nitrogen increased the digestibility of the cellulose from 17·8 to 38·7 per cent. They did not simultaneously study the influence of the urea supplement on the bacterial population of the rumen. However, data obtained at this Institute [van der Wath (1943)] show that the addition of urea to the type af basal ration employed by Harris and Mitchell does stimulate the proliferation of the paunch flora. Such an increase in the number of the organisms might conceivably have been responsible for the observed improvement in the digestion of the cellulose.

It has been found by one of us (J.G.v.d.W.) that the bacterial count in the rumen of sheep grazing on the natural veld of the Transvaal Highveld fluctuated with the changing condition of the pasture during the various seasons of the year. The highest count was found to occur during the summer months when the pasture is young, succulent, and of high nutritive value; the lowest count, on the other hand, was observed during the winter months when the veld is dry, fibrous, and of low nutritive value. Winter grazing in the Transvaal frequently has a protein content as low as 3·0 per cent. Conceivably, such a low protein content may limit the proliferation of the organisms in the rumen to such an extent that the breakdown of the cellulose cannot take place to the limit assumed to be set by the degree of lignification of the pasturage. The result would be that the animal is unable to utilise all the available energy-yielding nutrients of the winter grazing which is already deficient in protein and energy.

The experiments described below aimed primarily at ascertaining the influence of small supplements of protein-rich and carbohydrate-rich foodstuffs on the bacterial count in the rumen and on the digestibility of the

cellulose in a ration of winter grazing containing approximately 3·0 per cent. of protein. Secondly, the carbohydrate-rich supplement was gradually increased to determine the effect on the microbiotic decomposition of the cellulose in the winter grazing. The nitrogen metabolism of the animals was studied in all the trials conducted.

Experimental.

The experiment consisted of a series of cellulose and nitrogen balance studies on five full-grown merino wethers with closed rumen fistulae and ranging from 75 to 130 lb. in body weight. Veld hay containing approximately 3·0 per cent. of protein, the type of feed which is representative of winter grazing, served as the basal ration and was fed in period 1. In period 2 each animal received daily in addition to the basal ration a quantity of meatmeal which was taken to be sufficient to rectify the deficit in the protein requirement for maintenance on the basis that an animal weighing 100 lb. requires about 24·0 grams of digestible protein per day for maintenance [c.f. Smuts and Marais (1939)]. From periods 3 to 7 the ration fed in period 2 was further supplemented with increasing amounts of crushed maize. The allowance of meatmeal was, however, reduced with each addition of maize in order to ensure a more or less constant intake of digestible protein from period 2 onwards. In period 7 when 300 grams of maize were fed and in the case of some of the lighter animals in earlier periods this level of protein intake was, however, more or less exceeded. During all the periods, including period 1 on the basal ration, each sheep received daily 3 grams of yeast, 5 grams of bone-ash, and 5 grams of common salt. Full details of the ration are given in Table 1. Unfortunately only two of the five sheep could be used in the first three periods. However, these periods were repeated with all five animals after the comple ion of period 7.

A preliminary feeding period of 10 days was allowed throughout except in the case of the repetition of period 3 following period 7 when the preliminary period lasted 15 days. Collection periods were of 10 days' duration. The Forbes type of metabolism cage was employed. The faeces and the urine were collected daily, the usual procedures for collecting, preserving, and aliquoting being followed. Feeds, faeces, and urine were analysed for total nitrogen by the usual Kjeldahl method. The method of Norman and Jenkins (1933) was employed to determine the cellulose in feeds and faeces. Samples of the ruminal ingesta were withdrawn through the fistulae on at least three consecutive days during a collection period for the bacterial counts and the average of such counts taken, the Petroff-Hanser counting chamber method described by van der Wath (1943) being used. The animals were fed twice daily, at 9 a.m. and 3 p.m., and the samples for the bacterial counts were withdrawn in the morning immediately before feeding.

Results.

(a) *Cellulose Digestion.*

The essential collection data are given in Table 1. Periods 1, 2, and 3 were repeated after the conclusion of period 7 in the order shown in the table. The discussion of the results will be based on the average values obtained in the seven periods in which five sheep were employed. The cellulose and protein contents of the feeds are presented in Table 2 while the coefficients of digestibility for cellulose together with the figures for the bacterial counts, representing millions of bacteria per cubic centimetre of ruminal ingesta, are given in Table 3.

In the light of a statistical analysis of the relevant data, which demands a difference of 169 between two means for significance at $P = \cdot 01$, inspection of the averages for the five sheep given in the last column of Table 3 reveals two major changes in the bacterial count for consecutive periods of the experiment. The addition of the small amount of meatmeal to the basal ration resulted in a highly significant increase in the bacterial count from 1229 in period 1 (basal ration) to 1582 in period 2 (basal ration and meatmeal). The supplementation of the ration of period 2 with increasing amounts of crushed maize caused only minor increases in the bacterial count for consecutive periods up to and including period 5 for which a count of 1826 was obtained. A small decrease in the count to 1782 in period 6 was followed by a significant drop to 1566 in period 7, coinciding with an increase in the maize supplement from 200 to 300 grams per day (c.f. Table 1). It was observed that whereas for all other periods the bacterial counts made on consecutive days remained more or less constant a pronounced drop in the daily count occurred towards the end of period 7. Thus the counts for sheep 5 were 1760, 1445, 1225, and 1206 for the last four days of this period, respectively. Apparently, therefore, conditions in the rumen were most favourable for bacterial proliferation during periods 3, 4, and 5. The increase in the density of the bacterial population from periods 1 to 5 was associated with a progressive enrichment of the basal ration with highly digestible protein (meatmeal) and carbohydrate (maize). On the other hand further increases in the carbohydrate-rich supplement created conditions which became progressively less favourable to total bacterial growth. Towards the end of period 7 when the sudden decrease in the bacterial count was noted, the hydrogen ion concentration in the ruminal ingesta was determined and found to vary between 5·6 and 6·4 for the five animals. Although no pH determinations for any of the other periods were undertaken for comparison with these values there can be little doubt that the observed acidity was unfavourable to the growth of at least some of the bacteria.

Turning now to the average values for the digestibility of the cellulose given in the last column of Table 3 it is evident that the initial increases in the *number* of bacteria from periods 1 to 5 were not accompanied by an improvement in the digestibility of the cellulose. In fact, the tendency was rather in the opposite direction indicating an inverse relationship between the digestibility of the cellulose and the bacterial count for the first five periods of the experiment. In order to test the significance of the difference between the means for the coefficients of digestibility for cellulose in the seven periods of the experiment in which five sheep were employed the procedure for the well-known analysis of variance was applied. From this analysis it was established that the necessary difference between means for significance should be 3·30 for $P = \cdot 05$, and 4·51, for $P = \cdot 01$. Calculating from Table 3 it is found that the difference between the means for periods—

1 and 7 = 7·12, i.e., significant at $P = \cdot 01$.
2 and 7 = 3·68, i.e., significant at $P = \cdot 05$.
3 and 7 = 5·00, i.e., significant at $P = \cdot 01$.
4 and 7 = 3·30, i.e., significant at $P = \cdot 05$.
5 and 7 = 3·64, i.e., significant at $P = \cdot 05$.
1 and 6 = 5·86, i.e., significant at $P = \cdot 01$.
2 and 6 = 2·52, not significant.
3 and 6 = 3·74, i.e., significant at $P = \cdot 05$.
1 and 5 = 3·48, i.e., significant at $P = \cdot 05$.
1 and 4 = 3·82, i.e., significant at $P = \cdot 05$.

Differences not specified in the above summary were not significant. The general tendency for the digestibility of the cellulose was to decrease gradually from period 1 to 7. That being so, it may be expected that if the difference between periods 3 and 6 is significant, that between periods 2 and 6 should show even greater significance. In reality the difference between the latter two periods was found to be insignificant. At this juncture no feasible explanation can be offered for this finding except that it may be in the sequence of the seven periods on which the statistical analysis was made—periods 3, 2, and 1 following in this order after period 7. Inspection of the individual coefficients of digestibility reveals that all five animals digested the cellulose less efficiently in period 2 (basal ration + meatmeal) than in either period 3 (basal ration + meatmeal + maize) or period 1 (basal ration).

However that may be, the salient feature in the results remains that the digestibility of the cellulose in the hay, representing poor winter grazing, was not improved by supplementing the hay with varying amounts of meatmeal and crushed maize in spite of an increase in the number of bacteria in the rumen—the site of cellulose digestion.

At this stage it should, however, be pointed out that the method employed yields figures for the total number of bacteria in the ruminal ingesta. The organisms counted were not all of the same kind and it is conceivable that some of the species were not essential in the process of cellulose digestion. The number of the organisms actually responsible for the breakdown of the cellulose molecule may have undergone no change at all, it may have increased, or it may even have decreased with the modifications in the rations fed in periods 1 to 7. Whatever the case may be, it seems warranted to infer that the ingestion of the basal ration, representing, approximately, the nutritive value of winter grazing in the Transvaal, created conditions in the rumen of the sheep sufficiently favourable to the existence of that number of organisms of the right kind necessary for the maximum utilization of the cellulose in the ration. If that be so, then the degree of lignification, which, as pointed out earlier, is known to influence cellulose digestion, seems to be an important if not the only factor governing the magnitude of the breakdown of the cellulose in pasturage containing about 3·0 per cent. protein. The qualification as to protein content (N x 6·25) is considered pertinent in view of the recent finding of Harris and Mitchell (loc. cit.), previously referred to, viz., that the digestion of the cellulose in a N-low ration can be improved by supplementing such a ration with a nitrogenous substance. As stated, the basal ration, employed by these workers had a nitrogen content of only 0·136 per cent. or 0·85 per cent. protein. Van der Wath (loc. cit.), found that the bacterial count in the rumen of sheep on a basal ration containing 0·3 per cent. nitrogen increased from 612 to 1068 when the basal ration was supplemented daily with 2·33 grams nitrogen in the form of urea, and from 612 to 1875 when a similar daily supplement of nitrogen was given in the form of white fishmeal. The influence of these increases on cellulose digestion was, unfortunately, not studied. Nevertheless, van der Wath's results in conjunction with the results obtained by Harris and Mitchell seem to justify the conclusion that the total number of ruminal bacteria as determined in this investigation will constitute a second limiting factor in the digestion of the cellulose in rations very low in nitrogen content, in addition to the well-known influence of the degree of lignification of the plant material.

The depressing influence of the greater maize supplements on the digestibility of the cellulose in the basal ration has previously been indicated. The magnitude of this depression in digestibility was governed by the size of the supplement so that the odds in favour of the significance of the depression increased generally as the supplements of maize increased. The digestibility of the total dry matter improved on an average from 47·5 per cent. in period 1 to 60·7 per cent. in period 7, corresponding with an increasing amount of highly digestible maize in the total dry matter eaten (c.f. Table 1). Inspection of the mean values given in the last column of Table 1 reveals, however, that the dry matter of the veld hay alone, which contributed practically the whole of the cellulose in the supplemented rations, was digested in a manner similar to the cellulose in periods 1 to 7, decreasing from 47·5 per cent. in period 1 to 36·1 per cent. in period 7, These coefficients which were calculated on the assumption that the maize was completely digestible somewhat exaggerate the depressing influence of the added maize on the digestibility of the hay. Odd pieces of undigested maize have, for instance, occasionally been detected in the faeces, especially in that collected in periods 6 and 7 when the heavier supplements were given. Nevertheless, such a depression in digestibility is in agreement with a fact established by numerous experiments (c.f. Armsby, 1917). The effect is most distinct when pure digestible carbohydrates, such as starch, cane sugar, etc., are added, but manifests itself also when large amounts of feeding stuffs rich in carbohydrates are introduced. In the latter case it is, however, often impossible to follow the quantitative relations clearly. Thus, in the experiment under discussion it is not possible to determine what proportion of the total faecal dry matter was derived from the maize and what from the veld hay. However, the undigested cellulose present in the faeces may be taken to be derived almost exclusively from the veld hay, due to the very small contribution of the maize to the total intake of this constituent. For this reason the magnitude of the depression on the digestibility of the dry matter of the veld hay, caused by the maize supplements, seems to be best reflected in the coefficients of digestibility obtained for cellulose.

To conclude this discussion reference may be made to the possible influence of the supplement necessary for the rectification of the known deficiencies for maintenance in winter grazing, mentioned in the introduction, on the utilization of its available energy. Smuts and Marais (loc. cit.) inferred from a series of metabolism studies with sheep that 150 grams of maize daily will successfully supplement the protein and energy deficiencies for maintenance in winter grazing. The basal ration of poor veld hay was supplemented with this amount of maize in period 5 of the present investigation. Reference to Table 3 reveals that the 150 grams of maize depressed the digestibility of the cellulose in the basal ration from 65·5 to 62·1 per cent. Although the odds in favour of this difference being significant was found to be 100:5 the actual depression in digestibility may be considered to be of no practical importance in the energy metabolism of the animal.

(b) *Nitrogen Metabolism.*

The data relating to the nitrogen metabolism of the five sheep during the seven periods of the experiment are presented in Table 4. The mean values on which the following brief discussion is based are given in the last column of the table.

In period 1 on the basal ration the daily nitrogen intake amounted to 2·48 grams of which only 0·23 gram was apparently digested. The animals were definitely not receiving sufficient nitrogen for maintenance as was evidenced by the pronounced negative balance of 1·03 grams nitrogen per day. A protein deficiency of this nature has been shown to prevail for almost six months of the year in certain areas of the Union of South Africa [c.f. du Toit *et al* (1940) and Smuts and Marais (1940)]. In period 2 the total daily nitrogen intake increased to 5·87 grams, mainly due to the supplement of meatmeal. The nitrogen apparently digested increased tenfold to 2·31 grams, but in spite of this the nitrogen balance remained negative at 0·93 grams per day. From period 2 to period 6 the daily nitrogen intake remained practically the same, the small increases being mainly due to the slightly higher nitrogen content of the hay consumed. The nitrogen apparently digested increased relatively more, from 2·31 grams in period 2 to 2·85 grams in period 6. The small increases in digestible nitrogen intake was accompanied by marked changes in the manner of its utilization. Thus, while the amount of nitrogen excreted in the faeces remained more or less the same, fluctuating between 3·33 and 3·63 grams daily, that excreted in the urine decreased from 3·24 grams daily in period 2 to 1·90 grams daily in period 6. Simultaneously the daily nitrogen balance changed from −0·93 in period 2 to +0·95 in period 6, i.e., from a relatively strong negative to a relatively strong positive nitrogen balance. Reference to Table 4 shows that meatmeal nitrogen was gradually replaced by nitrogen derived from maize in the rations fed from periods 2 to 6. From this it may be inferred, either that the protein of meatmeal was not as efficiently utilized as that of maize, or that portion of the protein was catabolized for energy purposes especially in period 2. The effect of both these metabolic processes would be that less protein was available for fulfilling the nitrogen requirements for maintenance. The first-mentioned possibility is, however, ruled out by the biological values obtained for the proteins of the two foodstuffs, viz., 67·0 for maize [Marais and Smuts (1940)] and the same figure, 67·0, for meatmeal [du Toit and Smuts (1941)]. If, at the same time, it is remembered that the gradual replacement of meatmeal nitrogen by maize nitrogen was unavoidably accompanied by the introduction of an increasing amount of highly digestible carbohydrate to the rations fed in periods 2 to 6 (c.f. Table 1), then the only feasible explanation for the observed changes in the nitrogen utilization seems to be that an energy deficiency existed in at least the ration of period 2. The increasing supplements of maize from period 3 onwards gradually eliminated the energy deficiency with the result that the available nitrogen could more and more be utilized for its primary function in metabolism, viz., the replenishment of the unavoidable nitrogen losses associated with the minimum metabolism of the protoplasm, and tissue growth.

Similar results have been obtained by Smuts and Marais (loc. cit.). They found, for instance, that when winter grazing was supplemented with 56·0 grams of peanutmeal per day the sheep were in a considerably negative nitrogen balance, in spite of the fact that the nitrogen intake was raised to 6·5 grams daily by the supplement. On the other hand, in another trial where straw replaced the winter grazing, the daily allowance of peanutmeal reduced to 32·0 grams, and sufficient energy provided in the form of dextrinized starch, the sheep were found to be on the whole in nitrogen equilibrium with a daily nitrogen intake of only 3·5 grams. The conclusion drawn by these workers, viz., that " under practical conditions it will be

futile to rectify the existing protein deficiency with a minimum quantity of protein unless the energy requirements are (simultaneously) satisfied " is strongly supported by the results of the present investigation.

Summary.

From the results of a series of metabolism studies on sheep with open rumen fistulae in which a basal ration of winter grazing was supplemented with meatmeal and increasing amounts of crushed maize it was found that:—

(1) Small amounts of meatmeal and supplements of maize ranging from 50 grams to approximately 150 grams per day favoured the growth of the rumen organisms. Heavier supplements of maize, on the other hand, tended to reduce the number of organisms in the rumen.

(2) The increase in the bacterial count did not improve the digestibility of the cellulose in the winter grazing. A progressive depression in its digestibility with increasing supplements of maize was, however, observed.

(3) The rectification of the existing protein deficiency in winter grazing with a minimum quantity of protein is futile unless its energy deficiency is simultaneously satisfied.

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TABLE 1.

Showing Collection Data and Digestibility of Dry Matter.

Period.	Sheep No.	Weight (lb.).		Daily Ration (Gm.).			Average Daily Orts.* (gm. Dry Matter).	Average Daily Dry Matter Intake (gm.).		Average Daily Dry Matter Excreted. (gm.).	Coefficient of Digestibility for dry matter.			
		Initial.	Final.	Hay.	Maize.	Meat-meal.		Hay.	Total.		Total.	Average.	Hay.	Average.
1	3	100	99	600	0	0	33·0	538·0	538·0	293·4	45·5	—	45·5	—
	5	135	133	600	0	0	11·0	561·0	561·0	279·4	50·2	—	50·2	—
2	3	99	97	600	0	25	21·4	532·4	556·2	289·1	48·0	—	45·7	—
	5	129	127	600	0	34	14·5	539·3	571·6	286·8	49·8	—	46·9	—
3	3	93	94	600	50	17	21·2	544·8	606·5	299·1	50·7	—	45·1	—
	5	125	125	600	50	28	8·8	557·2	629·4	280·0	55·6	—	49·8	—
4	3	95	95	600	100	10	3·9	551·1	651·8	306·3	53·0	51·9	44·4	42·6
	4	108	109	600	100	16	45·0	510·0	616·4	281·3	54·4		44·8	
	5	125	128	600	100	20	1·0	554·1	664·3	290·8	56·2		47·6	
	6	92	86	600	100	10	18·5	536·5	637·2	314·0	50·8		41·5	
	7	75	74	600	100	4	59·5	495·5	590·5	324·8	44·9		34·5	
5	3	95	96	600	150	4	13·0	539·0	679·6	306·2	55·0	56·0	43·2	43·9
	4	112	110	600	150	7	66·8	485·2	628·6	249·6	60·3		48·6	
	5	132	131	600	150	12·5	0	552·0	700·7	270·0	61·5		51·1	
	6	85	87	600	150	4	41·6	510·4	651·0	300·9	53·8		41·1	
	7	76	78	600	150	0	55·0	497·0	633·8	321·3	49·4		35·4	
6	3	96	96	600	200	0	76·0	476·0	658·4	279·7	57·5	56·8	41·2	41·0
	4	110	111	600	200	1·5	79·9	472·1	655·9	265·3	59·6		43·8	
	5	131	132	600	200	7·0	3·7	548·3	737·3	304·6	58·7		44·4	
	6	88	87	600	200	0	33·1	518·9	701·3	313·9	55·3		39·5	
	7	79	77	600	200	0	39·4	512·6	695·0	328·8	52·7		35·9	

* Orts were composed of hay only.

TABLE 1.—(*continued*).

Period.	Sheep No.	Weight (lb.). Initial.	Weight (lb.). Final.	Daily Ration (Gm.). Hay.	Daily Ration (Gm.). Maize.	Daily Ration (Gm.). Meat-meal.	Average Daily Orts.* (gm. Dry Matter).	Average Daily Dry Matter Intake (gm.). Hay.	Average Daily Dry Matter Intake (gm.). Total.	Average Daily Dry Matter Excreted. (gm.).	Coefficient of Digestibility for dry matter. Total.	Coefficient of Digestibility for dry matter. Average.	Coefficient of Digestibility for dry matter. Hay.	Coefficient of Digestibility for dry matter. Average.
7	3	97	96	600	300	0	166·0	383·0	656·6	239·8	63·5		37·4	
	4	112	113	600	300	0	192·0	357·0	630·6	230·8	63·4		35·4	
	5	135	136	600	300	0	33·6	515·4	789·0	289·0	63·4	60·7	44·0	36·1
	6	91	88	600	300	0	76·0	473·0	746·6	316·5	57·6		33·1	
	7	79	78	600	300	0	60·1	488·9	762·5	340·6	55·4		30·4	
3† (Rep.).	3	99	97	600	50	16	20·2	524·6	585·4	282·9	51·6		46·0	
	4	110	110	600	50	19	109·0	435·8	499·4	221·2	55·7		49·3	
	5	128	127	600	50	27	5·4	539·4	610·6	285·9	53·2	50·7	47·0	44·7
	6	86	86	600	50	15	25·6	519·2	579·0	309·2	46·6		40·5	
	7	78	76	600	50	12	23·5	521·3	578·3	309·2	46·6		40·7	
2 (Rep.)	3	99	95	600	0	24	26·5	521·3	544·1	288·0	47·0		44·8	
	4	109	106	600	0	25	119·1	428·7	452·4	230·6	49·1		46·2	
	5	128	121	600	0	33	75·6	472·2	503·6	269·4	46·5	45·0	43·0	42·4
	6	87	83	600	0	21	30·8	517·0	537·0	323·8	39·7		37·4	
	7	77	76	600	0	18	28·1	519·7	536·8	308·2	42·6		40·7	
1 (Rep.)	3	89	90	600	0	0	107·5	438·5	438·5	232·3	47·0		47·0	
	4	102	104	600	0	0	122·6	423·4	423·4	203·5	51·9		51·9	
	5	117	117	600	0	0	105·5	440·5	440·5	233·1	47·1	47·5	47·1	47·5
	6	81	83	600	0	0	54·3	491·7	491·7	265·1	46·1		46·1	
	7	83	75	600	0	0	37·5	508·5	508·5	278·2	45·3		45·3	

† Repetition.

TABLE 2.

Showing Percentage Composition of Feeds (Dry Matter Basis).

Feed.	Constituent.	PERIODS.									
		1	2	3	4	5	6	7	3 (Rep.).	2 (Rep.).	1 (Rep.).
Hay......	Cellulose.....	51·3	52·3	50·4	49·9	48·8	48·6	48·4	48·7	48·7	49·5
	Crude protein	3·00	3·24	3·11	3·55	3·49	3·41	3·42	3·16	3·16	3·00
Maize....	Cellulose.....						1·8				
	Crude protein						11·0				
Meatmeal.	Crude protein						80·0				
Yeast....	Crude protein						50·0				

TABLE 3.

Showing Coefficients of Digestibility for Cellulose with the Ruminal Bacterial Counts in Brackets.*

Period.	Sheep 3.	Sheep 4.	Sheep 5.	Sheep 6.	Sheep 7.	Means.
1............	62·9 (1148)	—	68·1 (926)	—	—	—
2............	66·2 (1844)	—	67·4 (1696)	—	—	—
3............	63·7 (1864)	—	68·8 (1900)	—	—	—
1 (Repetition)..	65·4 (1200)	71·4 (1189)	65·2 (1156)	63·6 (1244)	62·1 (1356)	65·5 (1229)
2 (Repetition)..	63·2 (1511)	66·7 (1644)	64·7 (1711)	56·6 (1500)	59·8 (1542)	62·2 (1582)
3 (Repetition)..	65·3 (1644)	69·5 (1756)	67·7 (1722)	57·1 (1622)	57·5 (1767)	63·4 (1702)
4............	63·9 (1889)	65·3 (1789)	67·7 (1566)	59·6 (1839)	52·1 (1844)	61·7 (1785)
5............	61·9 (1785)	67·6 (1907)	70·5 (1700)	58·2 (1781)	52·1 (1948)	62·1 (1826)
6............	59·3 (1800)	64·5 (1944)	66·4 (1744)	57·1 (1733)	51·1 (1689)	59·7 (1782)
7............	58·9 (1517)	61·2 (1622)	67·7 (1408)	54·0 (1644)	50·3 (1641)	58·4 (1566)

* Figures represent millions of bacteria per c.c. ruminal contents.

TABLE 4.

Nitrogen Metabolism of Sheep in Periods 1 to 7 (1 to 3 were Repetitions).

Period.		Sheep 3.	Sheep 4.	Sheep 5.	Sheep 6.	Sheep 7.	Average for 5 Sheep.
1 (Rep.)	Nitrogen intake :—						
	Hay........................	2·01	2·23	2·02	2·45	2·51	2·24
	Yeast......................	·24	·24	·24	·24	·24	·24
	TOTAL............	2·25	2·47	2·26	2·69	2·75	2·48
	Nitrogen outgo :—						
	Faeces......................	2·11	2·07	2·18	2·41	2·50	2·25
	Urine......................	1·01	1·41	1·41	1·27	1·18	1·26
	TOTAL............	3·12	3·48	3·59	3·68	3·68	—
	N.—Balance..................	—0·87	—1·01	—1·33	—0·99	—0·93	*—1·03*
	N.—(Apparently) digested.......	0·14	0·40	0·08	0·28	0·25	0·23
	%N.—(Apparently) digested.....	6·2	16·2	3·5	10·4	9·1	9·1

TABLE 4.—(*continued*).

Period		Sheep 3.	Sheep 4.	Sheep 5.	Sheep 6.	Sheep 7.	Average for 5 Sheep.
2 (Rep.)	Nitrogen intake :—						
	Hay	2·64	2·38	2·39	2·61	2·63	2·53
	Yeast	·24	·24	·24	·24	·24	·24
	Meatmeal	3·07	3·20	4·22	2·69	2·30	3·10
	TOTAL	5·95	5·82	6·85	5·54	5·17	5·87
	Nitrogen outgo :—						
	Faeces	3·56	3·17	3·77	3·78	3·50	3·56
	Urine	2·88	3·60	3·99	2·94	2·80	3·24
	TOTAL	6·44	6·77	7·76	6·72	6·30	—
	N.—Balance	−0·49	−0·95	−0·91	−1·18	−1·13	*−0·93*
	N.—(Apparently) digested	2·39	2·65	3·08	1·76	1·67	2·31
	%N.—(Apparently) digested	40·2	45·6	45·0	31·8	32·3	39·0
3 (Rep.)	Nitrogen intake :—						
	Hay	2·65	2·39	2·73	2·63	2·64	2·61
	Yeast	·24	·24	·24	·24	·24	·24
	Meatmeal	2·05	2·43	3·45	1·92	1·53	2·27
	Mealies	·81	·81	·81	·81	·81	·81
	TOTAL	5·75	5·87	7·23	5·60	5·22	5·93
	Nitrogen outgo :—						
	Faeces	3·30	2·88	3·75	3·37	3·33	3·33
	Urine	1·88	3·04	3·00	2·22	2·18	2·46
	TOTAL	5·18	5·92	6·75	5·59	5·51	—
	N.—Balance	+0·57	−0·05	+0·48	+0·01	−0·29	*+0·14*
	N.—(Apparently) digested	2·45	2·99	3·48	2·23	1·89	2·61
	%N.—(Apparently) digested	42·6	50·9	48·2	39·8	36·2	43·5
4	Nitrogen intake :—						
	Hay	3·13	3·00	3·15	3·05	2·95	3·06
	Yeast	·24	·24	·24	·24	·24	·24
	Meatmeal	1·28	2·05	2·56	1·28	·51	1·53
	Mealies	1·61	1·61	1·61	1·61	1·61	1·61
	TOTAL	6·26	6·90	7·56	6·18	5·31	6·44
	Nitrogen outgo :—						
	Faeces	3·62	3·64	3·77	3·57	3·55	3·63
	Urine	2·16	3·37	3·09	2·71	2·17	2·70
	TOTAL	5·78	7·01	6·86	6·28	5·72	—
	N.—Balance	+0·48	−0·11	+0·70	−0·10	−0·41	*+0·11*
	N.—(Apparently) digested	2·64	3·26	3·79	2·61	1·76	2·81
	%N.—(Apparently) digested	42·2	47·3	50·2	42·3	33·1	43·0

TABLE 4.—(*continued*).

Period.		Sheep 3.	Sheep 4.	Sheep 5.	Sheep 6.	Sheep 7.	Average for 5 Sheep.
5	Nitrogen intake:—						
	Hay	3·00	2·86	3·08	2·94	2·90	2·96
	Yeast	·24	·24	·24	·24	·24	·24
	Meatmeal	·51	·90	1·60	·51	0	·70
	Mealies	2·42	2·42	2·42	2·42	2·42	2·42
	TOTAL	6·17	6·42	7·34	6·11	5·56	6·32
	Nitrogen outgo:—						
	Faeces	3·60	3·24	3·53	3·47	3·52	3·47
	Urine	1·64	2·17	2·63	2·17	1·68	2·06
	TOTAL	5·24	5·41	6·16	5·64	5·20	—
	N.—Balance	+0·93	+1·01	+1·18	+0·47	+0·36	+*0·79*
	N.—(Apparently) digested	2·57	3·18	3·81	2·64	2·04	2·85
	%N.—(Apparently) digested	41·7	49·6	51·9	43·2	36·7	44·6
6	Nitrogen intake:—						
	Hay	2·46	2·76	3·00	2·84	2·80	2·77
	Yeast	·24	·24	·24	·24	·24	·24
	Meatmeal	0	·19	·90	0	0	·22
	Mealies	3·22	3·22	3·22	3·22	3·22	3·22
	TOTAL	5·92	6·41	7·36	6·30	6·26	6·45
	Nitrogen outgo:—						
	Faeces	3·29	3·51	3·92	3·60	3·68	3·60
	Urine	1·48	1·99	2·77	1·71	1·54	1·90
	TOTAL	4·77	5·50	6·69	5·31	5·22	—
	N.—Balance	+1·15	+0·91	+0·67	+0·99	+1·04	+*0·95*
	N.—(Apparently) digested	2·63	2·90	3·44	2·70	2·58	2·85
	%N.—(Apparently) digested	44·5	45·2	46·8	42·9	41·2	44·1
7	Nitrogen intake:—						
	Hay	2·04	2·37	2·81	2·75	2·80	2·55
	Yeast	·24	·24	·24	·24	·24	·24
	Mealies	4·84	4·84	4·84	4·84	4·84	4·84
	TOTAL	7·12	7·45	7·89	7·83	7·88	7·63
	Nitrogen outgo:—						
	Faeces	3·58	3·67	3·97	4·23	4·40	3·97
	Urine	1·55	1·98	2·11	1·74	1·76	1·83
	TOTAL	5·13	5·65	6·08	5·97	6·16	—
	N.—Balance	+1·99	+1·80	+1·81	+1·86	+1·72	+*1·84*
	N.—(Apparently) digested	3·54	3·78	3·92	3·60	3·48	3·66
	%N.—(Apparently) digested	49·7	50·7	49·7	46·0	44·2	48·1

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Grass Hay as a Maintenance Ration for Sheep During Winter.

By J. G. LOUW, Section Biochemistry and Nutrition, Onderstepoort.

THE deficiencies of the winter grazing of the summer rainfall areas of the Union have often been stressed in publications from this Institute. Investigations [du Toit *et al* (1940); Smuts and Marais (1940); Louw and van der Wath (1943)] have shown that apart from the well-known phosphorus deficiency there also exists an acute shortage of protein and of energy. These last-mentioned deficiencies, energy probably more than protein, may be considered to be the primary cause of the highly emaciated condition of stock on pasture alone during winter. In this connection it may be of interest to refer to the finding [Louw (1938)] that grass which has grown undisturbed during the season until early in April contained only about 50 per cent. of the available energy present in grass at the grazing stage of growth. Dry winter pasture contains probably less available energy than the grass cut early in April.

As a result of the deficiencies of the winter pasture the animal is forced to draw upon its own body substance in order to provide the nutrients for carrying on a variety of internal processes which are essential to life. According to Smuts and Marais (loc. cit.) sheep husbandry can be established on a sound basis only when this breakdown of tissue which has been synthesized during summer is prevented by applying a judicious and economical method of supplementary winter feeding. If future research should prove this view to be essentially correct then the problem will resolve itself into providing the animal with a maintenance ration during the dry winter months. This object may be achieved by supplementing the available grazing with a feed such as maize, as suggested by Smuts and Marais (loc. cit.). The utilization of the excess summer growth of our grass pastures for the purpose of providing a maintenance ration for stock during winter is an alternative which could with advantage be explored, in view of the fact that this excess growth is in any case wasted if not preserved in a nutritious state. From the results of an investigation previously referred to [Louw (loc. cit)] it was concluded that veld grass should be cut at the flowering stage of growth for the purpose of preserving it in the form of hay to be used as a feed for winter or other times of food scarcity such as drought.

The object of the investigation to be reported on in this paper was to determine the amount of hay, from grass cut at the flowering stage of growth, necessary for the maintenance requirements of sheep during winter.

EXPERIMENTAL DETAILS.

The grass hay used was kindly provided by the Division of Soil and Veld Conservation from its Research Station at Rietondale, Pretoria. It was made from a pure stand of the Zeerust strain of *Digitaria*, harvested

at the flowering stage of growth. This hay was fed to a group of six full-grown Merino wethers, as their sole ration, in amounts just sufficient to maintain live weight. The sheep were shorn op April 9, and feeding of the experimental hay commenced on the next day. They were kept for the whole of the experimental period in separate feeding pens with concrete floors and measuring about (16 by 5) square feet, of which a third is under cover. In a preliminary feeding period lasting until May 26, the amounts of hay consumed were varied in accordance with changes in the body weights of the animals with the object of establishing the level of intake which would ensure approximately constant body weights. No difficulties were encountered in getting the animals to consume the required amounts of hay. In fact, the orts were for all the animals at no time more than about 4 per cent. of the total amount of hay offered and consisted of inedible material, such as the coarse woody stems of weeds and grass stubble.

The experimental feeding started on May, 26, and lasted until August, 6—a period of ten weeks. The animals were weighed once a week and a record kept of their food intake. Towards the end of the experimental period five of the sheep were placed in metabolism cages of the Forbes type and their faeces and urine collected for a period of 10 days. In addition to the usual analyses the gross energy contents of the feed, faeces, and urine were determined in a bomb calorimeter. The calorific value of the urine was obtained by evaporating to dryness at room temperature a total of 10·0 c.c. of urine, 2·0 c.c. at a time, on a weighed piece (about 1·5 grams) of filter paper of known calorific value and determining the gross energy content of the paper plus urine after it has been briquetted. These energy determinations and the computation of the methane output of the sheep from Armsby's factor of 4·5 grams of methane per 100 grams of digestible carbohydrates made an estimation of the metabolizable energy of the grass hay possible.

The Results.

The weekly weights of the individual sheep are given in Table 1. Inspection of the data shows that all the sheep maintained their body weights at an approximately constant level from May, 26, to August, 6.

Table 1.

Body Weight Records (in lb.) during Maintenance Experiment.

Date.	Sheep 1.	Sheep 2.	Sheep 3.	Sheep 4.	Sheep 5.	Sheep 6.
1942—						
May 26.....	82·5	75·0	76·0	72·0	74·5	77·5
June 2.....	82·0	74·0	77·0	71·0	74·0	76·0
„ 9.....	82·0	73·0	76·0	71·0	74·5	77·0
„ 16.....	83·0	73·5	75·5	71·0	74·5	75·5
„ 23.....	84·0	76·5	77·5	72·0	75·0	78·0
„ 30.....	82·0	76·0	77·5	72·0	73·0	79·0
July 7.....	83·0	75·0	78·0	71·0	74·0	77·0
„ 14.....	84·5	76·5	77·0	71·5	75·0	78·0
„ 21.....	81·5	74·0	74·5	70·0	75·0	77·0
„ 31.....	83·0	75·0	76·0	71·0	75·0	78·0
Aug. 6.....	82·5	72·0	74·0	70·5	74·0	76·5
Average.......	82·7	74·6	76·3	71·2	74·4	77·2

Small variations in weight did occur from week to week but there was no tendency for the weights of the sheep either to increase or to decrease progressively.

From the average coefficients of digestibility for the grass hay and its average chemical composition the average content of the hay in total and digestible nutrients was calculated, the results being given in Table 2. The computation of the metabolizable energy of the grass hay will be found in Table 3. It will be seen that on an average 41·7 per cent. of the energy consumed was metabolizable. This figure agrees very well with that of 42·6 for timothy hay as determined by American workers with steers in the respiration chamber where the loss of energy due to methane can be actually determined [see Mitchell *et al* (1928)].

TABLE 2.

Average Percentage Composition of the Grass Hay.

	Dry Matter.	Organic Matter.	Crude Protein.	Crude Fat.	Carbohydrates.*	Total Digestible Nutrients	Ash.	P.
Total.................	93·2	86·2	7·64	2·98	75·6	—	7·01	0·114
Digestible.............	50·4	47·5	4·33	1·48	41·8	49·46	—	—

* Carbohydrates = crude fibre + nitrogen-free extract.

TABLE 3.

Calculation of the Metabolizable Energy of the Hay.

Sheep No.	Dry Matter Consumed. (lb.).	Energy of Feed Consumed. (Therms).	Energy of Faeces. (Therms).	Energy of Urine. (Therms)	Energy of Methane. * (Therms)	Energy† Correction for Nitrogen Balance (Therms)	Total Metabolizable Energy. (Therms).	Metabolizable Energy as Per cent. of Gross Energy.	Metabolizable Energy per lb. Dry Matter Consumed. (Therms).
1	1·453	2·885	1·416	·121	·173	·003	1·172	40·6	·806
2	1·482	2·941	1·402	·116	·181	·006	1·236	42·0	·834
3	1·491	2·959	1·406	·128	·184	·005	1·236	41·8	·829
4	1·491	2·959	1·379	·125	·187	·006	1·262	42·7	·846
6	1·502	2·981	1·434	·128	·183	·005	1·231	41·3	·819
Average	—	—	—	—	—	—	1·227	41·7	·827

* One gram methane contains 13·34 Calories of gross energy.

† (7·45 × N—balance) Calories.

It is now possible from the figures for the average daily consumption of hay during the maintenance experiment and the data presented in Tables 1, 2, and 3 to make the final calculations of the experiment. This is done in Table 4. The maintenance weight of the individual sheep is simply the average of all the weekly weights in Table 1. The average daily ration

given in column 3 of Table 4 refers to the amount of hay consumed during the period covered by the average maintenance weights. The metabolizable energy consumed daily (see column 4) was obtained by using the average result of the metabolism trial with five sheep. In the last two columns of Table 4 the average daily intake of feed and of metabolizable energy has been computed to 100 lb. body weight, using the ratio of the weights to the three-fourths power.

TABLE 4.

Maintenance Requirements of Sheep per 100 lb. Live Weight.

Sheep No.	Maintenance Weight. (lb.).	Average Daily Ration. (lb.).	Metabolizable Energy per Day (Cals.).	Average Daily Ration per 100 lb. Live Weight.	
				Hay. (lb.).	Metabolizable Energy. (Cals.).
1	82·7	1·720	1,325	1·984	1,528
2	74·6	1·687	1,300	2·102	1,619
3	76·3	1·710	1,317	2·094	1,614
4	71·2	1·710	1,317	2·206	1,699
5	74·4	1·727	1,330	2·155	1,661
6	77·2	1·725	1,328	2·094	1,613
Average	—	—	—	2·106	1,622

The nitrogen and phosphorus balances were also determined in the 10-day metabolism trial. All the sheep were found to be in positive nitrogen balance; the daily balance in grams nitrogen were 0·40, 0·82, 0·72, 0·81 and 0·62 for sheep Nos. 1, 2, 3, 4 and 6, respectively. In the same order for the 5 sheep the phosphorus balances were in grams P per day −0·072, +0·018, +0·015, +0·003, and −0·031.

DISCUSSION OF RESULTS.

According to the data in Table 4 a full-grown Merino wether weighing 100 lb. require daily 2·106 lb. hay, containing 1622 Calories of metabolizable energy, for maintenance during winter. As stated previously, this figure represents the average daily consumption of hay during a period in which the animals maintained their weight at an approximately constant level. Live-weight as the sole criterion in measuring the nutritive requirement for maintenance may be criticised on the ground that the constancy of weight does not necessarily imply the maintenance of the integrity of the body tissues. However, in the case of adult animals such as those employed in this experiment it may be assumed that a fairly accurate measure directly applicable to the conditions of practice is obtainable by the constant live-weight procedure.

The results obtained in this investigation with the hay from the *Digitaria* species cut at the flowering stage of growth agree well with data secured in experiments on similar lines by Mitchell *et al* (1926, 1928) in which the energy value of lucerne hay for the maintenance of sheep has been investigated. For western ewes, three to four years of age, they found

that 1·917 lb. of lucerne hay containing 1864 calories of metabolizable energy were needed for maintenance per 100 lb. live weight. In another trial in which the experimental animals were western lambs, about 10 months of age, and in which a slaughter test was included, the daily requirements for maintenance per 100 lb. body weight were found to be 2·185 lb. of lucerne hay containing 1733 Calories of metabolizable energy. From several live-weight experiments on sheep Armsby (1917) computed their metabolizable energy requirement for maintenance at 1624 Calories per 100 lb. live-weight, a figure which is practically the same as that reported in this paper (cf. Table 4).

An interesting feature emerging from the above comparison of results is that a good quality grass hay such as that used in this experiment has more or less the same energy value as lucerne hay for the maintenance of sheep. This does not mean that lucerne hay may not be superior to the grass hay in other respects. It contains, for instance, more than twice as much protein as the grass hay. However, the non-nitrogenous energy value of lucerne is apparently so low that part of its protein has to serve as a source of energy in a maintenance ration.

Judging from the results of the metabolism trial the grass hay supplied slightly more protein than was necessary for maintenance. In the case of phosphorus the daily balances indicate that the animals were ingesting just about sufficient of this nutrient for their maintenance requirements.

Subject to the conditions under which this experiment has been conducted it may be concluded that hay, from grass cut at the flowering stage of growth, can successfully be used as a maintenance ratic ı for sheep during winter. No supplements such as bonemeal are needed. Mention must, however, be made of the fact that the opportunities for exercise were rather limited as the sheep were confined, as stated previously, to individual feeding pens measuring about 16 by 5 square feet. In addition, the sheep could during cold and windy nights make use of the shelter offered by the feeding pens. Both of these factors may result in a measure which is an underestimation of the maintenance requirements of sheep which have to roam about on the open veld for their feed supply. On the other hand, it was observed that during the latter half of the experimental period the sheep required about 7 per cent. less hay for the maintenance of constant body weight than during the first half. The explanation for this phenomenon may be somewhat as follows: Exposure of animals to cold air temperatures increases the loss of heat by radiation, especially if they have scanty coats. The heat lost in this way must be made good by the energy of its food if the animal is to maintain its live-weight at a constant level. The sheep used in this experiment were shorn, as stated, under "experimental details", on April, 9, forty-six days prior to the commencement of the experiment. It is conceivable, therefore, that during the latter half of the experiment, when their wool coats were thicker than at the start, the animals were able to maintain live weight on an amount of hay slightly less than the average for the whole experimental period, i.e., slightly less than the maintenance requirement as estimated in this investigation.

In any case, if a grass hay is to be used for the maintenance of sheep during winter it will probably be fed to them under conditions which will, in total effect, not differ markedly from those obtaining during the experiment reported in this paper. The data obtained have shown that from the

nutritional point of view it is possible to utilize the excess growth of the summer months successfully for the prevention of weight losses in sheep during winter. The economic feasibility of such a scheme remains to be investigated under practical conditions.

Summary.

Constant live-weight has been used as criterion in measuring the maintenance requirement of adult sheep during winter. It has been found that an animal weighing 100 lb. requires 2·106 lb. of a grass hay, containing 1622 Calories of metabolizable energy, to maintain its weight at an approximately constant level.

Acknowledgment.

The author wishes to record his indebtedness to Dr. J. C. Fick and Mr. C. J. J. van Rensburg of the Division of Soil and Veld Conservation for kindly providing the hay used in this experiment.

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Lantanin, the Active Principle of *Lantana camara L.* Part I.—Isolation and Preliminary Results on the Determination of its Constitution.

By P. G. J. LOUW, Section of Toxicology and Pharmacology, Onderstepoort.

Lantana camara is a very popular ornamental plant especially grown in hedges. It is not indigenous to South Africa but has been introduced from America. It is widely spread along the Natal coast. In the course of investigations by Steyn and Van der Walt (1941) into the disease occurring among dairy cattle near Durban, poisoning caused by this plant was suspected. The symptoms of poisoning by *Lantana camara* are very similar to those described by Quin (1933) in cases of poisoning with *Lippia rehmanni* and *Lippia pretoriensis*.

The toxicity of the plant obtained from the Durban area was duly investigated (Steyn and Van der Walt, 1941) and a sample of the dry leaves was submitted to the author for chemical investigation.

Extraction.

From preliminary experiments the following general method of extraction has been adopted.

Five hundred (500) gm. of the dry powdered leaves of the plant in the flowering and seeding stages were extracted at room temperature with 1,000 ml. 96 per cent. alcohol. After filtration the alcohol solution was clarified with activated charcoal. A light-brown filtrate resulted. This filtrate was concentrated to about 500 ml. by evaporating the alcohol by means of a fan when spontaneous crystallization took place. The yield was approximately 0·3 per cent. per dry weight of leaves. After repeated re-crystallizations from 96 per cent. alcohol it melted at 276-280° C. with decomposition. The amount of this compound in different samples of plant investigated varied considerably, viz., from 0·31 to 0·68 per cent. per dry weight of powdered leaves.

The name suggested for this substance is "Lantanin".

Fig. 1. Lantanin. M.P. 276-280°. (×50).

*Micro-Analysis of Lantanin.

	%*C*	%*H*	*Mol. Wt. Rast.*
Found	75·06	8·97	516
Calculated for $C_{31}H_{44}O_5$	74·96	8·93	497

Two (2) gm. of Lantanin dosed *per os* to a sheep which was then exposed to direct sunlight, caused photosensitization and a severe icterus similar to that observed by administering the plant.

Properties of Lantanin.

(1) Lantanin crystallises in prismatic needles from 96 per cent. alcohol. It is colourless, tasteless and odourless.

(2) It is insoluble in water but very soluble in ether, chloroform, carbon tetrachloride, benzene, pyridine, acetone, ethylacetate, methanol, petroleum ether, glacial acetic acid and acetic anhydride.

(3) It is insoluble in hot concentrated HCl.

(4) It is insoluble in hot dilute sulphuric acid but dissolves in concentrated sulphuric acid with an orange colour. On gentle heating the solution becomes red and afterwards carbonization occurs.

(5) It is insoluble in 25 per cent. nitric acid, but dissolves in 65 per cent. nitric acid with a yellow colour. A yellow precipitate forms on cooling.

* All the micro-analyses by Dr. Backeberg of the University of the Witwatersrand, Johannesburg, to whom I am very much indebted.

(6) An alcoholic solution gives no colour reaction with ferric chloride.

(7) Dilute alkaline potassium permanganate solution is decolourised, especially on warming.

(8) Dissolved in carbon tetrachloride, Lantanin decolourises Bromine-water.

(9) Tests for nitrogen were negative.

(10) Ignited on a platinum disc, Lantanin burns with a very smoky flame leaving no residue.

Optical Activity.

151·8 mg. Lantanin was dissolved in 8 ml. chloroform and the rotation, using a 10 cm. tube, determined

Rotation	$=1 \cdot 72^\circ$
Blank	$=0 \cdot 06^\circ$
$\therefore \theta$	$=1 \cdot 66^\circ$
$\therefore [\alpha]_D^{20^\circ}$	$=\dfrac{1 \cdot 66 \times 1000 \times 8.}{151 \cdot 8 \times 1}$
	$= +80 \cdot 74$ ($CHCl_3$).

No change of the rotation was observed when left overnight.

Determination of the Oxygen Functions of Lantanin.

I. *Lactone Grouping.*

Lantanin exhibits no acid action.

(i) To 115 mg. Lantanin, 10·ml. $\frac{N}{10}$ $\times 0 \cdot 9852$ was added and refluxed for 3 hours and the excess NaOH titrated.

ml. $\frac{N}{10}$ NaOH added $= 9 \cdot 85$
ml. $\frac{N}{10}$ NaOH back-titrated $= 9 \cdot 75$

It is therefore evident that Lantanin is not an acid nor an ester.

(ii) 50 mg. Lantanin was dissolved in 10 ml. 96 per cent. alcohol and then titrated at room temperature with $\frac{N}{10}$ alcoholic KOH using phenolpthalein as indicator.

ml. $\frac{N}{10}$ KOH used $= 1 \cdot 04$.

Theoretical for one lactone group in 50 mg. Lantanin $= 1 \cdot 004$ ml. $\frac{N}{10}$ KOH.

It is therefore evident that the Lantanin molecule contains a lactone group, accounting for two oxygen atoms.

Molecular weight.—From the titration value the molecular weight of the substance was calculated to be 480.

Theoretical for $C_{31}H_{44}O_5$: 497.

Relactonisation of Hydroxy-acid.—50 mg. Lantanin dissolved in 10 ml. alcohol and titrated with $\frac{N}{10}$ alcoholic KOH required

1·04 ml.

On addition of 5 ml. $\frac{N}{10}$ HCl the lactone was immediately reformed. From the solution Lantanin with M.P. 276—280° C. was recovered. Mixed melting point with pure Lantanin gave no depression.

It is therefore evident that the lactone is easily titrated at room temperature with alcoholic potash and that the hydroxy-acid is immediately relactonized with hydrochloric acid which is characteristic of γ-lactones.

Isolation of the potassium salt of the saponified lactone.—One (1) gm. of Lantanin was dissolved in excess alcoholic potash solution. The excess KOH was neutralized with hydrochloric acid and the mixture evaporated to dryness. The residue was extracted with ethyl acetate. On evaporation of the solvent 1 gm. of feathery crystals were obtained. After repeated crystallization from ethyl acetate it melted sharply at 229° C. with decomposition.

The potassium salt is insoluble in water; soluble in alcohol, methanol, chloroform, acetone, ether, benzene and ethyl acetate.

A sample of the potassium salt was digested with sulphuric acid and the potassium determined by precipitation as potassium cobaltinitrite and the potassium determined titrimetrically.

Analysis :—

	%*K*.
Found	6·59, 6·55
Calculated for $C_{31}H_{45}O_6K$	7·07

II. *Ketone Group.*

(*a*) *Preparation of semi-carbazone.*—0·2 gm. of Lantanin dissolved in 25 ml. absolute alcohol was refluxed with 0·2 gm. semi-carbazone hydrochloride and 0·2 gm. anhydrous sodium acetate dissolved in 1 ml. water. After ¾ hour ±0·2 gm. of a white crystalline material separated which upon repeated crystallization from absolute alcohol gave a M.P. 285° C., with decomposition.

Micro-analysis :—

	C%	*H*%	*N*%
Calculated for $C_{32}H_{47}O_5N_3$	69·59	8·55	7·59
Found	70·31	8·62	7·66

It is therefore evident that a ketone group exists in the Lantanin molecule and the above further confirms the empyrical formula of Lantanin.

(*b*) *Preparation of the 2:4 dinitrophenylhydrazone.*—0·2 gm. Lantanin was dissolved in 30 ml. absolute alcohol and 0·2 gm. 2:4 dinitrophenylhydrazine dissolved in 5 ml. absolute alcohol was mixed and 2 ml. 25 per cent. HCl added. 0·2 gm. orange-coloured prismatic needles crystallized after 15 minutes.

After repeated recrystallization from absolute methanol, the crystals melted sharply at 268° C.

Micro-analysis :—

	C%	H%	N%
Found	66·72	7·43	8·74
Calculated $C_{37}H_{48}O_{8}N_{4}$	65·66	7·15	8·27

III. *Hydroxy Groups.*

(*a*) *Methylation of Hydroxy Groups in Lantanin.*—To 10 ml. of a cold saturated solution of Lantanin in methyl alcohol (containing approximately 0·3 gm. Lantanin), 2 ml. nitroso-n-methyl-urethane was added and then 10 ml. of a cold saturated solution of KOH in methyl alcohol added and gently mixed in an icebath.

The mixture was left overnight and then evaporated to dryness. After the addition of distilled water a white substance separated out which was filtered, washed and recrystallized from absolute alcohol. After repeated recrystallizations it melted at 208-213° C. and was negative for nitrogen.

Micro-analysis :—

	C%	H%
Found	75·95	9·07
Calculated for 2 hydroxy groups, viz., $C_{33}H_{48}O_{5}$	75·53	9·22

(*b*) *Preparation of the acetylderivative.*—1·0 gm. Lantanin was refluxed for 3 hours with 3 ml. of acetic anhydride and 0·5 gm. anhydrous sodium acetate.

The mixture was then poured into ice-water when 0·9 gm. of a white substance separated out. It was filtered, washed and repeatedly crystallized from 96 per cent. alcohol, when it melted with decomposition at 246-250° C.

Refluxing 88 mg. of the acetylderivative for 6 hours with 10 ml. $\frac{N}{10}$ alcoholic potash, 3·52 ml. $\frac{N}{10}$ KOH was used.

ml. $\frac{N}{10}$ KOH used for saponification of the lactone group in 88 mg. acetyl derivative = 1·77

∴ ml. $\frac{N}{10}$ KOH used for the saponification of the acetyl group in 88 mg. acetyl derivative = 1·75.

Theoretical value for 1 acetyl group = 1·68 ml.

This indicates that one hydroxy group was acetylated.

Micro-analysis :—

	C%	H%
Found	75·73	9·06
Calculated for one hydroxy group [$C_{31}H_{43}O_{4}(OCOCH_{3}) - H_{2}O$]	76·01	8·51

From the micro-analysis it would appear that acetylation took place with the loss of one molecule of water. This would explain the difference in the number of hydroxy groups established by means of methylation and acetylation.

Ethylenic Double Bonds.

Preliminary qualitative tests showed that Lantanin was unsaturated towards Bromine-water and Potassium permanganate.

Using 96 per cent. acetic acid as solvent and platinum dioxide as catalyst, 5 gm. Lantanin was hydrogenated until the hydrogen absorption was complete. A blank experiment using the same amount of PtO_2 as before was conducted and it was found that 495 ml. hydrogen had been absorbed at 21° C. and 644 mm. pressure.

Vol. H_2 absorbed (reduced to N.T.P.) = 389·5 ml.

Theoretical vol. for 5 gm. Lantanin for one double bond (at N.T.P.) = 225 ml.

Two double bonds of Lantanin have therefore been hydrogenated.

Separation of the tetra-hydro-derivative.—After hydrogenation the PtO_2 was filtered off. On standing the tetra-hydro-derivative crystallized in transparent needles. After further recrystallizations from 96 per cent. alcohol it sublimes at 237° C. and melts at 261-4° C. with decomposition.

Micro-analysis :—

	%C	%H
Found ...	73·74	9·60
Calculated $C_{31}H_{48}O_5$ ...	74·31	9·65

As soon as more plant material is available it is hoped to conduct degradation of the Lantanin molecule by oxidation and dehydrogenation.

Summary.

1. The photosensitising constituent of *Lantana camara L.* has been isolated and named *Lantanin*. The empyrical formula is $C_{31}H_{44}O_5$.

2. The functions of the five oxygen atoms have been determined, viz., a lactone group, a keto group and two hydroxy groups.

Acknowledgements.

Many thanks are due to Drs. Steyn and de Waal for their interest and guidance.

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The Isolation of the Toxic Principle "Potassium Cymonate" from "Gifblaar" *Dichapetalum cymosum* (Hook) Engl.

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It has long been known that "Gifblaar" *Dichapetalum cymosum* (*Chailletia cymosa*) is one of the most poisonous plants of Southern Africa. The plant is mainly distributed throughout the Northern and Western Transvaal, Bechuanaland and South-West Africa. A good summary of our present knowledge in respect of poisoning by Gifblaar is given by Steyn (1928) with reference to various attempts to isolate the toxic principle. In 1935 Rimington reported on the chemical investigation of the plant giving an account of the different methods employed by him in endeavouring to isolate the toxic principle.

In the present investigation the methods described by Rimington were used to prepare a concentrate of the toxin. By further manipulations and purification it was found possible to prepare an amorphous hygroscopic powder which was highly toxic to rabbits, viz., 5 mgm. per kilogram body-weight. On investigating this concentrate it proved to contain some sodium acetate. In attempts to remove the acetate by liberating the acetic acid it was observed that the toxic principle behaved in very much the same way as the acetic acid. Thereupon the methods of extraction were further investigated and modified until the following was evolved as the most suitable method of extraction.

Method for the Isolation of the Toxic Principle.

Ten kilograms of the dried and finely ground plant material are continuously extracted with 96 per cent. alcohol for 36 to 48 hours in a large soxhlet extraction apparatus. After extraction, the alcohol is distilled off under diminished pressure. The extract, which now consists of a syrupy mass, containing a large amount of chlorophyll, is taken up in about 4 litres of water and after acidifying with 500 c.c. of a 10 per cent. sulphuric acid solution, it is left to stand for a day or two, to allow the chlorophyll and other precipitated material to settle. The solution is then filtered, using a large buchner filter and applying suction. This gives a clear although slightly brown coloured filtrate. After dividing in suitable portions the filtrate is repeatedly shaken out with ether. The ether shakings are neutralized with N potassium hydroxide, using phenolphthalein as indicator. By this means the acid extracted by the ether is converted into the potassium salt, which is insoluble in the ether and goes, therefore, into the water solution. After separation of the potassium salt solution from the ether extract, the ether solution is distilled to remove dissolved chlorophyll. Thus the recovered

ether may be used over and over again for shaking out the acid filtrate. This process of shaking out with ether and neutralizing with potassium hydroxide is continued until only negligible amounts of acid are extracted by the ether.

The combined potassium salt solution is concentrated to about 300 c.c. by distilling off under diminished pressure on a waterbath. The free acid is again liberated by adding the equivalent amount of dilute sulphuric acid. This solution is now repeatedly shaken out with ether until only negligible amounts of acid are extracted by the last ether shakings, as tested by neutralizing an aliquot against $\frac{N}{10}$ potassium hydroxide solution, using phenolphthalein as an indicator. The total ether shakings are decolorized by activated charcoal and dried overnight by adding anhydrous sodium sulphate. The sodium sulphate and charcoal are filtered off and the ether solution concentrated by gently distilling off the ether. As soon as nearly all the ether has been distilled off, the concentrate is rinsed with small amounts of ether into a small distillation flask and again distilled, collecting in fractions of 10 degrees up to 160° C. At this stage only a small viscid dark brown residue, which hardens on cooling, remains in the distillation flask. All the fractions as well as the initial ether distillate are now carefully neutralized with N potassium hydroxide using phenolphthalein as indicator. The potassium salt solutions are now separately evaporated to dryness on a waterbath. The residues are washed with acetone, dried at 100° C. and crystallized from 96 per cent. alcohol. The lower boiling fractions yield only small amounts of crystals and the ether distillate none at all. The best yields of the crystalline potassium salt are obtained from the fractions 110°–160° C. All of the crystalline material is collected, pooled and further purified by recrystallization from 96 per cent. alcohol. The name suggested for this crystalline potassium salt is potassium cymonate.

Experimental Results.

Using the above described method for the extraction of 10 kilograms of plant material, the initial ether shakings required altogether 600 c.c. N potassium hydroxide ($f = 0{\cdot}83$). After again acidifying with the equivalent amount of sulphuric acid it was found necessary to shake out 16 times with ether before all of the acid had been extracted. The yields of potassium cymonate from the different fractions were as follows:—

Fraction.	c.c. N. KOH (f = 0·83), required for neutralization.	Yield of Crystalline K salt	M.P. of Crystalline K salt
1. Ether distillate 40°	31·0 c.c.	None	—
2. 40°– 50°	1·8 c.c.	0·0280 gm.	185°–203°
3. 50°– 60°	1·5 c.c.		
4. 60°– 90°	4·0 c.c.		
5. 90°–100°	5·5 c.c.	0·2586 gm.	200°–208°
6. 100°–110°	17·5 c.c.		
7. 110°–120°	14·2 c.c.	0·7534 gm.	200°–213°
8. 120°–130°	21·5 c.c.		
9. 130°–140°	24·4 c.c.	0·6000 gm.	206°–213°
10. 140°–150°	24·7 c.c.	0·8270 gm.	208°–213°
11. 150°–160°	46·7 c.c.	1·1870 gm.	208°–213°
	Total.......	3·6540 gm.	

After the removal of the potassium cymonate the residues of the different fractions were investigated. It was found that fraction 1 contained mostly potassium formate mixed with a small amount of potassium acetate, whilst the higher fractions contained potassium formate, potassium acetate and the potassium salts of higher boiling unidentified acids. After repeated recrystallization of the potassium cymonate from 96 per cent. alcohol it melted at 213° C. with decomposition and a colour change from yellow to red. The purity of the potassium cymonate was tested by recrystallizing five times from 96 per cent. alcohol. No change in melting point could be obtained.

Potassium cymonate is insoluble in anhydrous organic solvents excepting methyl alcohol. It dissolves very easily in water and is soluble with difficulty in 96 per cent. alcohol.

Attempts to isolate the free acid in a purified state failed since slight decomposition occurred when it was distilled. The potassium salt itself gave unreliable combustion analysis, so that with regard to the nature of this substance very little can be said at present.

These investigations will be continued as soon as more plant material becomes available.

Toxicity of the Potassium Cymonate.

The crystalline potassium cymonate proved to be very toxic to rabbits and gave rise to the typical gifblaar poisoning with the same general post-mortem appearances. The results of the dosing experiments are summarized in the following table:—

Rabbit.	Weight in Kilograms.	Dosed.	Mgm. K Cymonate per Kilogram.	Result.
1	1·9	Per os............	11·5	Died within 1 hour 30 minutes.
2	2·1	,, ...	3·2	Died within 1 hour 20 minutes.
3	1·9	,, ...	1·9	Died within 1 hour 10 minutes.
4	2·0	,, ...	1·0	Died within 11 hours.
5	2·0	,, ...	0·75	Died within 3 hours 15 minutes.
6	2·3	,, ...	0·75	Died within 8 hours.
7	2·1	,, ...	0·75	Died within 26 hours 45 minutes.
8	2·2	,, ...	0·50	Died within 3 hours 5 minutes.
9	2·4	,, ...	0·50	Died within 6 hours 45 minutes.
10	2·0	,, ...	0·50	Died within 12 hours 45 minutes.
11	2·6	,, ...	0·50	Died overnight.
12	1·8	,, ...	0·50	Died overnight.
13	2·4	,, ...	0·50	Recovered.
14	2·0	,, ...	0·50	Recovered.
15	2·1	,, ...	0·50	Recovered.
16	2·1	,, ...	0·25	Recovered.
17	2·2	,, ...	0·25	Recovered.
18	2·0	,, ...	0·25	Recovered.
19	1·8	,, ...	0·25	Recovered.
20	3·3	Intravenously......	0·25	Recovered.
21	1·5	,, ,, ...	0·25	Recovered.
22	1·8	,, ,, ...	0·25	Recovered.
23	2·2	,, ,, ...	0·25	Recovered.

Rabbit.	Weight in Kilograms.	Dosed.	Mgm. K Cymonate per Kilogram.	Result.
24	2·1	Intravenously......	0·50	Died within 43 minutes.
25	3·3	,, ,, ...	0·50	Died within 2 hours 13 minutes.
26	1·9	,, ,, ...	0·50	Recovered.
27	1·6	,, ,, ...	0·50	Recovered.
28	1·5	,, ,, ...	0·75	Died within 3 hours 56 minutes.
29	1·8	,, ,, ...	0·75	Died overnight.
30	2·2	,, ,, ...	0·75	Died overnight.
31	3·3	Subcutaneously.....	0·25	Died overnight.
32	2·8	,, ,, ...	0·25	Recovered.
33	2·1	,, ,, ...	0·25	Recovered.
34	1·7	,, ,, ...	0·25	Recovered.
35	1·7	,, ,, ...	0·50	Died within 1 hour 38 minutes.
36	2·4	,, ,, ...	0·50	Died overnight.
37	2·8	,, ,, ...	0·50	Died overnight.
38	2·1	,, ,, ...	0·50	Recovered.
39	2·1	,, ,, ...	0·75	Died within 2 hours 40 minutes.
40	1·7	,, ,, ...	0·75	Died overnight.

It is apparent from these results that the M.L.D. for the rabbit is 0·5 to 0·75 mgm. of the potassium cymonate per kilogram bodyweight. It is also of interest to note that the M.L.D. *per os* is the same as that when administered intravenously or subcutaneously.

Summary.

A method for the isolation of potassium cymonate, the toxic principle of *Dichapetalum cymosum* has been described. The M.L.D. of potassium cymonate for the rabbit is 0·5 to 0·75 mgm. per kilogram bodyweight.

Acknowledgments.

I wish to express my sincere thanks to Dr. D. G. Steyn and Prof. H. L. de Waal for their keen interest throughout the course of the investigation.

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Recent Investigations into the Toxicity of Plants, etc., XIII.

By S. J. VAN DER WALT and DOUW G. STEYN, Section of Pharmacology and Toxicology, Onderstepoort.

AMARYLLIDACEAE.

Haemanthus magnificus Herb.

Registered number.—O.P.H. No. 20716; 30.3.42.

Common name.—Blood flower, seeroogblom.

Origin.—Queenstown, Cape Province.

State and stage of development.—The plant was in the fresh state without flowers or fruit.

Sheep 50431 (4-tooth; 29·6 Kg.) was given* 1·2 Kg. of the fresh bulbs and stems in the course of 6 hours.

Result.—Negative.

CAPPARIDACEAE.

Boscia foetida Schinz.

(See Fig. 1, page 222.)

Registered number.—O.P.H. No. 16153; 7.1.42. and 17599; 6.2.42.

Common name.—Noeniebossie, stinkbossie, oumiedbos.

Origin.—Upington, Cape Province.

State and stage of development.—The first consignment of the plant (O.P.H. No. 16153; 7.1.42.) was in the dry state and flowering stage whilst the second consignment (O.P.H. No. 17599; 6.2.42) was in the dry state and in the late flowering and early seeding stages.

Sheep.—59042 (4-tooth; 56·9 Kg.) was given 400 gm. of the leaves and flowers of the first consignment of the plant in the course of 6 hours.

Symptoms.—The animal died overnight on the day of drenching.

Post-mortem appearances.—Slight post-mortem changes; general cyanosis; hydrothorax; hydropericardium; ascites; subepicardial and subendocardial ecchymoses; regressive changes in the myocardium; hyperaemia and oedema of the lungs; tumor splenis; hyperaemia of,

* Except where otherwise stated, all the animals were drenched per stomach tube.

and regressive changes in, the liver; hyperaemia of the kidneys; tympanites of the rumen; severe hyperaemia of the mucous membrane of the rumen, reticulum, abomasum and duodenum; slight hyperaemia of the mucous membrane of the rest of the small intestine, caecum and colon.

Histology.—Moderate oedema and hyperaemia and vacuolar and nuclear degeneration of the liver; slight acute cholangitis; hypertrophy of the smooth muscle fibres of the spleen; hyperaemia and parenchymatous degeneration of the myocardium; hyperaemia and oedema of the lungs; hyperaemia of the kidney.

Sheep 54150 (6-tooth; 42·8 Kg.) was given 900 gm. of the leaves, flowers and immature fruit of the second consignment of the plant in the course of 24 hours.

Symptoms.—Apathy; anorexia; tympanites; inactivity of the rumen; dyspnoea; accelerated pulse; haemorrhagic diarrhoea. The sheep died two days after the commencement of dosing.

Post-mortem appearances.—General cyanosis; hydropericardium; ascites; pronounced hydrothorax; subendocardial ecchymoses; regressive changes in the myocardium; severe hyperaemia and oedema of the lungs; severe hyperaemia of, and regressive changes in, the liver; hyperaemia of the kidneys; calculi in the pelvis of the left kidney; hydronephrosis with calculi in the pelvis of the right kidney; hyperaemia of the mucous membrane of the reticulum; severe hyperaemia and slight oedema of the mucous membrane of the abomasum; severe hyperaemia of the mucous membrane of the small intestine and caecum; extensive haemorrhages in the mucous membrane of the caecum; hyperaemia of the mucous membrane of the rest of the large intestine.

Histology.—Severe hyperaemia, alveolar emphysema and atalectasis of the lungs; severe hyperaemia and parenchymatous degeneration of the myocardium; severe hyperaemia of the kidney; severe hyperaemia, fatty infiltration and necrobiosis of the liver; pronounced acute catarrhal typhlitis.

It is possible that uraemia was a contributary factor in causing death in sheep 54150.

Cadaba juncea (Linn.) B. and H.

Registered number.—O.P.H. No. 4904; 30.6.42.

Common name.—Swartstorm.

Origin.—Carnarvon, Cape Province.

State and stage of development.—The plant was in the dry state and in the pre-flowering stage.

Sheep 59763 (2-tooth; 34·1 Kg.) was given 800 gm. of the plant in the course of 7 hours.

Symptoms.—Tympanites; severe dyspnoea; accelerated pulse; frothing at the mouth. The animal died 12 hours after receiving the first dose.

Post-mortem appearances.—General cyanosis; pronounced ascites; hydrothorax and hydropericardium; subepicardial petechiae; regressive changes in the myocardium; emphysema, hyperaemia and oedema of the lungs; severe regressive changes in the liver and kidneys; tympanites of the rumen; slight hyperaemia of the mucous membrane of the small intestine; oedema of the omentum.

Histology.—Acute severe central necrotising hepatitis; parenchymatous degeneration of, and slight fatty changes in, the kidney; marked fatty degeneration and hyperaemia of the myocardium; hyperaemia and acute alveolar emphysema of the lungs.

Sheep 65304 (4-tooth; 29·1 Kg.) was given 400 gm. of the plant in one dose.

Symptoms.—Apathy; anorexia; tympanites; accelerated pulse; dyspnoea; bloodstained mucus in the nostrils. The animal died 30 hours after drenching.

Post-mortem appearances.—Advanced post-mortem changes; general cyanosis; ascites; hydrothorax; hydropericardium; subendocardial petechiae;; hyperaemia, emphysema and oedema of the lungs; tympanites of the rumen; oedema of the omentum and mesenterium; slight hyperaemia of the mucous membrane of the abomasum and caecum.

Sheep 60632 (full-mouth; 27.8 Kg.) was given 600 gm. of the plant in the course of 28 hours.

Result.—Negative.

COMPOSITAE.

Berkheyopsis echinus (Less.) O. Hoffm. and B. bechuanensis S. Moore.

Registered number.—O.P.H. No. 15834; 31.12.41. and 17695; 10.2.42.

Origin.—Baltimore, Transvaal.

State and stage of development.—The plant material was in the dry state and in the flowering stage. A specimen from the first consignment of the plant was identified as *B. echinus* whilst a specimen from the second consignment of the plant was identified as *B. bechuanensis.* Since both consignments were derived from the same farm it is probable that they consisted of a mixture of the two plants.

Sheep 59696 (2-tooth; 43·7 Kg.) was given 2·0 Kg. of the first consignment of the plant in the course of 30 hours.

Symptoms.—Apathy; anorexia; dyspnoea; accelerated pulse; rumen inactive; diarrhoea. The animal died 36 hours after the commencement of dosing.

Post-mortem appearances.—Advanced post-mortem changes; general cyanosis; hydrothorax; hydropericardium; subepicardial and subendocardial haemorrhages; hyperaemia and oedema of the lungs; severe hyperaemia of the trachea; severe tympanites of the rumen; slight hyperaemia of the mucosa of the small intestine; fluid material in the large intestine.

Sheep 50431 (full-mouth; 35·5 Kg.) was given 3·0 Kg. of the second consignment of the plant in the course of 5 days.

Result.—Negative.

Epaltes alata Steetz.

Registered number.—O.P.H. No. 18362 B-C; 27.2.42 and 18410; 2.3.42.

Origin.—Vredefort, Orange Free State.

State and stage of development.—The first consignment of the plant (O.P.H. No. 18362 B-C; 27.2.42) was fresh and in the flowering stage whereas the second consignment (O.P.H. No. 18410; 2.3.42) was almost dry and in the flowering stage.

Rabbit A (1·9 Kg.) was given 49 gm. of the first consignment of the plant in the course of 4 hours.

Result.—Negative.

Sheep 53396 (6-tooth; 49·1 Kg.) was given 1·2 Kg. of the second consignment of the plant in the course of 30 hours.

Symptoms.—Apathy; anorexia; icterus; enlarged abdomen; rumen inactive; dyspnoea; accelerated, weak pulse; constipation. The animal died 3½ days after the commencement of dosing.

Post-mortem appearances.—Advanced post-mortem changes; icterus; a tremendous number of petechiae throughout the body; hyperaemia and oedema of the lungs; hyperaemia of the mucous membrane of the caecum; stasis of the contents of the caecum and colon.

Othonna cluytiafolia O.K.

(See Fig. 2, page 223.)

Registered number.—O.P.H. No. 7315; 7.8.42.

Common name.—Ertjiebos.

Origin.—Carnarvon, Cape Province.

State and stage of development.—The plant was in the fresh state and in the flowering stage.

Sheep 53407 (4-tooth; 65·0 Kg.) was given 1·5 Kg. of the plant in the course of 24 hours.

Symptoms.—Apathy; anorexia; tympanites; dyspnoea; accelerated pulse. The animal died 36 hours after the commencement of dosing.

Post-mortem appearances.—A tremendous number of petechiae and ecchymoses throughout the body; hyperaemia and oedema of the lungs; regressive changes in the myocardium; advanced fatty degeneration of the liver; regressive changes in the kidneys; tumor splenis; haemorrhagic lymphadenitis of all the lymphatic glands; haemorrhagic abomasitis, enteritis, typhlitis and colitis.

Sheep 60331 (2-tooth; 19·6 Kg.) was given 500 gm. of the plant in the course of 7 hours.

Symptoms.—Apathy; anorexia; general weakness; tympanites; rumen inactive; weak pulse. The sheep died 28 hours after the commencement of dosing.

Post-mortem appearances.—General cyanosis, ascites; hydrothorax; hydropericardium; ecchymoses throughout the subcutis, salivary glands, peritracheal tissues, thymus and in the mucosa of the trachea; haemorrhagic lymphadenitis of all the lymphatic glands; subepicardial petechiae; regressive changes in the myocardium; "nutmeg" liver; severe regressive changes in the kidneys; severe hyperaemia and oedema of the lungs; tympanites of the rumen; slight hyperaemia of, and haemorrhages in, the mucous membrane of the abomasum; fairly marked hyperaemia of the mucous membrane of the small and large intestine.

Sheep 61269 (full-mouth; 31·0 Kg.) was given 1·5 Kg. of the plant in the course of 16 days.

Symptoms.—Apathy; anorexia; slight tympanites; rumen inactive; accelerated, weak pulse; dyspnoea. The symptoms developed from the eighth day after the commencement of dosing. The animal died 8 days later.

Post-mortem appearances.—Slight post-mortem changes; general cyanosis; ascites; hydrothorax; hydropericardium; generalized fat necrosis; a slight degree of icterus; severe hyperaemia and oedema of the lungs; slight regressive changes in the myocardium; pronounced enlargement and fatty degeneration of the liver; regressive changes in the kidneys; stasis of ingesta in the caecum and colon; slight hyperaemia of the mucosa of the colon.

Histology.—*Liver.*—If it was not for the presence of the portal tracts it would be difficult to identify the organ as liver. Microscopically it strongly resembles adipose tissue. There is practically no cellular reaction.

Lung.—Severe hyperaemia; agonal emphysema and extensive fat phagocytosis.

Kidney.—The cells, especially of the proximal convoluted tubules, contain numerous fat droplets of varying size and show necrobiotic changes. The cytoplasm of an occasional one of these cells has become finely granular and stains a bright eosinophilic colour. Even the cells of the collecting tubules contain fat droplets. There is a moderate hyperaemia of the kidney.

Adrenal.—There is an increased lipoid content of the cells of the adrenal cortex. The even distribution and size of these cells are, however, not indicative of degenerative changes. Fat laden phagocytes are absent. A number of cells do not contain fat, but a number of vacuoles without any indication of cellular degeneration.

Spleen.—The spleen contains numerous macrophages laden with fat.

Myocardium.—A slight degree of chronic interstitial myocarditis was observed.

Othonna pallens. D.C.

Registered number.—O.P.H. No. 3177; 29.5.41.

Common name.—Springbokbos.

Origin.—Bestersput, Orange Free State.

State and stage of development.—The plant was dry and in the post-seeding stage. In the following experiments only the leaves and tops of the plants were used.

Sheep 51158 (6-tooth; 45·0 Kg.) was given 200 gm. of the dry plant in the course of 30 hours.

Symptoms.—Apathy; anorexia; accelerated pulse; dyspnoea; rumen inactive. The animal died 4 days after the commencement of dosing.

Post-mortem appearances.—Cyanosis, hyperaemia and oedema of the lungs; subepicardial haemorrhages; fatty degeneration of the liver; fairly marked hyperaemia of the mucosa of the abomasum; stasis of the ingesta in the caecum and colon.

Histology.—Moderate peripheral fatty changes of the liver with considerable round cell infiltration of Glisson's capsule; haemosiderosis of the spleen; slight fatty changes of some tubules in the cortex of the kidney.

Sheep 54222 (8-tooth; 48·0 Kg.) was given 400 gm. of the plant in the course of 7 hours.

Symptoms.—The symptoms observed were similar to those of sheep 51158. The sheep died 60 hours after receiving the first dose.

Post-mortem appearances.—Advanced post-mortem changes; icterus; severe hyperaemia, oedema and emphysema of the lungs; emphysema of the subcutaneous tissues of the neck; pronounced regressive changes in the liver; stasis of ingesta in the caecum and colon.

Histology.—Well-marked, mainly central, fatty changes of the liver.

Each of *sheep* 54581 (6-tooth; 42·0 Kg.) and *sheep* 54150 (6-tooth; 45·0 Kg.) received 100 gm. of dry plant in one dose.

Symptoms.—Apathy; anorexia; accelerated, weak pulse, dyspnoea; rumen inactive; constipation. The animals appeared normal again on the ninth day after dosing.

Rabbit A (2·5 Kg.) was given 60 gm. of the plant in the course of 2 days.

Symptoms.—Listlessness; anorexia; dyspnoea. The rabbit died 3 days after the commencement of dosing.

Post-mortem appearances.—Cyanosis; severe hyperaemia and oedema and slight emphysema of the lungs; severe regressive changes in the liver; regressive changes in the kidneys; stasis of the ingesta in the colon.

Histology.—Necrosis and cytolysis of the central liver cells with round cell infiltration of this central area; marked fatty changes of the peripheral cells of the liver lobules; lymphocytic glomerulonephritis (early stages) and fatty changes of the epithelial cells of the renal tubules; fatty changes in the myocardium.

Rabbit B (2·1 Kg.) received 5·0 gm. of the dry plant daily from 15.9.41 to 2.10.41. From 22.9.41 the animal ate very little, was depressed, constipated and showed progressive loss of weight. It died on 3.10.41.

Post-mortem appearances.—Pronounced emphysema and slight hyperaemia of the lungs; fatty degeneration of the liver; very little ingesta in stomach and intestines.

Rabbit C (2·4 Kg.) received 10·0 gm. of the dry plant on 15.9.41 and again on 17.9.41. The animal recovered after having shown dyspnoea, anorexia and constipation for about a week after the second dose.

Rabbit D (2·45 Kg.) received 15·0 gm. of the dry plant on 15.9.41 and again on 17.9.41. The result was similar to that described in the case of Rabbit C.

Rabbit E (2·5 Kg.) received 20·0 gm. of the dry plant on 15.9.41 and again on 17.9.41. The animal died on 17.9.41 after having exhibited very severe dyspnoea and apathy.

Post-mortem appearances.—Pronounced general cyanosis; very severe oedema and hyperaemia of the lungs with slight emphysema; very marked fatty degeneration of the liver; stomach distended with ingesta; stasis of ingesta in large intestine; dilatation of both heart ventricles.

Histology.—Necrosis and cytolysis of the central liver cells with round cell infiltration of this area; marked fatty changes of the peripheral cells of the liver; early stages of nephritis and fatty changes in the epithelial cells of the renal tubules; fatty changes in the myocardium.

Platycarpha glomerata Less.

Registered number.—O.P.H. No. 15880; 3.1.42.

Origin.—Grahamstown, Cape Province.

State and stage of development.—The plant was in the flowering stage and almost dry.

Sheep 54150 (full-mouth; 49·1 Kg.) was given 1·0 Kg. of the above plant in the course of 6 hours.

Result.—Negative.

LEGUMINOSAE.

Gliricidia maculata H.B.K.

Registered number.—O.P.H. No. 7932; 13.8.42.

Origin.—Doornkop, Natal.

State and stage of development.—The plant was in the dry state without flowers or fruit.

Sheep 61184 (2-tooth; 23·7 Kg.) was given 2·4 Kg. of the leaves in the course of 4 days.

Symptoms.—Anorexia; listlessness; dyspnoea; weak pulse; rumen inactive; tympanites; diarrhoea. These symptoms developed on the second day after the commencement of dosing and persisted for 2 days, after which the sheep made a complete recovery.

Sheep 61388 (4-tooth; 31·4 Kg.) was given 6·6 Kg. of the leaves of the plant in the course of 13 days.

Result.—Negative.

Medicago sativa L.

Registered number.—O.P.H. No. 9459; 11.9.42.

Common name.—Lucerne.

Origin.—Delmas, Transvaal.

State and stage of development.—The lucerne hay was infected with the the following fungi: *Mucor* sp., *Penicilium* sp., *Helminthosporium* sp., and a *Fusarium* sp. probably *F. scirpi.*

Sheep 62032 (2-tooth; 18·7 Kg.) consumed 27·3 Kg. of the above hay in the course of 16 days.

Result.—Negative.

Phaseolus vulgaris L.

Registered number.—O.P.H. No. 7305; 5.8.42.

Common name.—van Zyl sugar bean; van Zyl suikerboontjie.

Origin.—Derby, Transvaal.

State and stage of development.—The material fed to two experimental horses was the same as that ingested by the affected horses, namely, very finely ground and fairly dusty hay mixed with a small percentage of the mature beans and shells of the pods. The hay was heavily infected with a *Macrosporium* sp. and *Fusarium moniliforme,* and to a lesser degree, with a *Mucor* sp., a *Penicilium* sp. and a *Cephalothecium* sp.*

Equine 63 (1 year) consumed 107·8 Kg. of the hay in the course of 32 days. The symptoms which developed were undoubtedly due to the fact that the animal consumed insufficient food to maintain life.

Symptoms.—The animal became emaciated and progressively weaker. Finally the eyelids and left hind limb became swollen. The fetlock of the right hind limb was also swollen and the animal was lame in this limb. The animal was discharged and placed on the usual laboratory diet; this resulted in rapid recovery.

Equine 22504 (3½ years) and *equine* 22502 (3½ years) consumed 364·0 Kg. of the hay in the course of 73 days, and 182·0 Kg. of the hay in the course of 30 days respectively. They developed no symptoms of illhealth. In spite of this negative result the sugar bean is still suspected as the circumstantial evidence incriminating it is very strong. Further investigations will be conducted as soon as the occasion arises.

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* The examination for the presence of fungi in the hay was kindly done by Mr. E. E. Schaefer, of the Division of Botany and Plant Pathology, Pretoria.

The above experiments were conducted as a result of a disease which broke out in horses in the Derby area and which was investigated by one of us (D. G. S.). The disease which had never been encountered previously, occurred in horses fed large quantites of the above hay. In previous years horses had not been extensively fed on this hay.

The following is a description of this disease, which can be divided into acute and chronic stages.

Symptoms: Acute stage.—Without any prodromal symptoms the horses develop acute brain symptoms. They usually stagger backwards until they assume a sitting position. They then jump up, look around in a wild manner and charge away through fences, bushes and over kraal walls. When they finally stop, completely exhausted, they walk in circles or up and down along fences in a dazed condition. At this stage repeated attacks of clonic convulsions shake the whole body, the eyes have a wild expression whilst the teeth are repeatedly bared as a result of clonic spasms of the lips. The conjunctivae are oedematous, icteric and show petechiae. The acute stage usually only lasts a few days.

Chronic stage.—The horses become emaciated and walk about in a state of semi-consciousness bumping into any object in their way. In standing, abnormal attitudes are assumed and food is often seen protruding from the mouth. Respiration is slow with a forced expiratory effort. The pulse is weak and slow and the animals are constipated. This stage of the disease resembles " dunsiekte " (Chronic Senecio poisoning) in horses.

Post-mortem appearances.—The following were observed in one animal (an eighteen-months-old horse) which was killed after having been affected for six weeks: Hydroperitoneum; dilatation of the stomach; cirrhosis of the liver; slight chronic catarrh of the distal portion of the small intestine; stasis in the colon; hyperaemia and oedema of the cerebral meninges.

Specimens of this young horse were collected for histological examination and these were kindly examined by Dr. G. de Kock, head of the Section of Pathology, and Dr. C. Jackson, head of the Section of Anatomy, Onderstepoort Laboratories. We quote hereunder their respective reports for which we thank them:—

(1) Dr. G. de Kock.—" *Liver:* Midzonal fatty changes, no evidence of much interference in the arrangements of the columns of liver cells with staining of the cells not quite clear, but no necrobiotic changes, their cytoplasm somewhat granular. Here and there in the periphery there is slight cellular proliferation. No undue proliferation of connective tissue.

Diagnosis.—Slight fatty changes.

Spleen.—Follicles and secondary follicles very prominent. In the red pulpa there is a fair amount of a brown granular pigment in the phagocytes. Here and there evidence of small haemorrhages."

(2) Dr. Jackson.—" *Embedded sections of brain:* Definite lesions are present only in the cerebrum. Here in the outer cell lamina there are extensive areas characterised by inflammatory softening, i.e., perivascular oedema, slight perivascular lymphocytic infiltration, necrosis of ganglion cells, multiplication and mobilisation of the glia cells; neuronophagia is demonstrable.

In the cerebellum there is diffuse degeneration of Purkinje cells without apparent reaction. No inclusions are seen; this implies also to the hippocampus.

Diagnosis.—Acute non-purulent encephalitis.

Remarks.—The detailed histological picture would be very atypical of Borna disease and no inclusions are found. Further, the distribution of the lesions as far as is seen from the sections available is not typical. While it is not possible to exclude Borna disease on findings like this in a single case, that diagnosis appears most unlikely."

Liliaceae.

Dipcadi sp. aff. D. viride Moench. and D. elatum Baker.

Registered number.—O.P.H. No. 17120; 27.1.42.

Origin.—Mafeking, Cape Province.

State and stage of development.—The plant was in the fresh state and in the late seeding stage.

Sheep 54150 (6-tooth; 43·7 Kg.) was given 2·3 Kg. of the plant in the course of 30 hours.

Result.—Negative.

Dipcadi sp. probably D. viride Moench.

Registered number.—O.P.H. No. 21703; 30.3.42.

Origin.—Deben, Cape Province.

State and stage of development.—The plant was in the fresh state and in the late seeding stage.

Sheep 60237 (4-tooth; 29·1 Kg) was given 1·4 Kg. of the bulbs in the course of 6 hours.

Result.—Negative.

Drimia alta R. A. Dyer, Nom. nov. (Drimia altissima Hook.)

Registered number.—O.P.H. No. 17040; 24.1.42.

Origin.—Pietermaritzburg, Natal.

State and stage of development.—The bulbs were in the fresh state without flowers or fruit.

Rabbit A (2·0 Kg.) was given 50 gm. of fresh bulbs in one dose.

Symptoms.—Dyspnoea; accelerated and strong pulse which subsequently became weak and irregular; paresis. The rabbit died 2 hours after drenching.

Post-mortem appearances.—General cyanosis; severe emphysema of the lungs; hyperaemia and regressive changes of the liver and kidneys; slight hyperaemia of the mucosa of the stomach and small intestine; slight dilatation of the stomach.

Rabbit B (2·0 Kg.) was given 25·0 gm. of fresh bulbs in one dose.

Symptoms.—As in the case of rabbit A. The rabbit died one hour after drenching.

Post-mortem appearances.—As in rabbit A.

Rabbits C (2·1 Kg.) and *D* (1·95 Kg.) received 50 gm. and 25 gm. of the fresh leaves respectively in one dose.

Symptoms and post-mortem appearances.—As in rabbit A.

Drimiopsis maculata Lindl.

Registered number.—O.P.H. No. 11844 A; 26.10.42.

Origin.—Port Shepstone, Natal.

State and stage of development.—The plant was fresh and in the flowering and seeding stages.

Sheep 61249 (4-tooth; 30·0 Kg.) was given 7·34 Kg. of the fresh plant in the course of 4 days.

Result.—Negative.

Scilla natalensis Planch.

Registered number.—O.P.H. No. 13726; 2.4.41.

Origin.—Estcourt, Natal.

State and stage of development.—The plant was in the fresh state and in the flowering stage.

Sheep 53394 (8-tooth; 65·5 Kg.) was given 1·2 Kg. of the fresh bulbs in one dose.

Symptoms.—Apathy; tympanites; severe dyspnoea; accelerated and weak pulse. Death occurred about twelve hours after drenching.

Post-mortem appearances.—Advanced post-mortem changes; general cyanosis; tympanites of the rumen; hyperaemia and oedema of the lungs.

Urginea macrocentra Baker.

Registered number.—O.P.H. No. 18263-64; 24.2.42.

Common name.—Natal slangkop.

State and stage of development.—The plant was in the fresh state and in the post-seeding stage.

Rabbit A (1·5 Kg.) was given 50 gm. of the fresh bulbs in one dose.

Symptoms.—The animal was found dead three hours after drenching.

Post-mortem appearances.—General cyanosis; hyperaemia and severe emphysema of the lungs; hyperaemia of, and regressive changes in, the liver and kidneys; hyperaemia of the mucous membrane of the stomach and small intestine.

OLEACEAE.

Jasminium angulare Vahl.

(See Fig. 3, page 224.)

Registered number.—O.P.H. No. 8219; 19.8.42.

Origin.—Elandskraal, Helpmekaar district, Natal.

State and stage of development.—The plant was fresh and in the post-seeding stage.

In the course of an investigation in the Helpmekaar district the plant was suspected of having caused serious mortality on quite a number of farms.

Sheep 62227 (4-tooth; 25·0 Kg.) was given 800 gm. of the fairly fresh leaves in the course of 4 hours.

Symptoms.—Apathy; general weakness; slight tympanites; accelerated pulse; dyspnoea. The sheep died 7 hours after receiving the first dose.

Post-mortem appearances.—General cyanosis; slight oedema and severe emphysema of the lungs; chronic intestinal catarrh.

Sheep 62077 (4-tooth; 28·8 Kg.) was given 1·2 Kg. of the dry leaves of the plant in the course of 24 hours.

Symptoms.—Apathy; slight tympanites; dyspnoea; accelerated pulse; diarrhoea. The animal died 25 hours after receiving the first dose.

Post-mortem appearances.—General cyanosis; slight hydropericardium, hydrothorax and ascites; hyperaemia and severe emphysema of the lungs; hyperaemia of, and regressive changes in, the liver; hyperaemia of the kidneys; intestinal catarrh; fluid material present in the large intestine.

Histology.—Severe hyperaemia of the lungs accompanied by acute alveolar emphysema and atalectasis; severe regressive changes of the liver accompanied by hyperaemia, oedema and bile pigmentation; moderate hyperaemia of the kidney; hyperaemia of the suprarenal cortex.

Bovine 9142 (1½ years) was given 3·0 Kg. of the fresh leaves of the plant in the course of 4 hours.

Symptoms.—Twelve hours after receiving the first dose the animal developed apathy, weakness in the hindquarters and dyspnoea. Twelve hours later the animal had recovered.

SANTALACEAE.

Thesium lineatum L.

Registered number.—O.P.H. No. 4905; 30.6.42.

Common name.—Vaalstorm, witstorm.

Origin.—Carnarvon, Cape Province.

State and stage of development.—The plant was dry and in the pre-flowering stage.

Sheep 64513 (4-tooth; 30·5 Kg.) was given 6·4 Kg. of the dry plant in the course of 9 days.

Result.—Negative.

SOLANACEAE.

Datura arborea L.

Registered number.—O.P.H. No. 17694; 9.2.42.

Origin.—Bonnefoi, Transvâal.

State and stage of development.—The plant was in the flowering stage and almost dry.

Rabbit A (1·85 Kg.) was given 10 gm. of the dry leaves in one dose.

Symptoms.—After drenching the rabbit vomited and developed severe dyspnoea. The rabbit died overnight on the day of drenching.

Post-mortem appearances.—The post-mortem changes were too far advanced to allow of the detection of any lesions that may have been present.

Rabbit B (1·6 Kg.) was given 15 gm. of the dry leaves in the course of 24 hours.

Result.—Negative.

THYMELEACEAE.

Gnidia caffra Meisn. forma pulchra (B. Davy). M. Moss, M.S.

Registered number.—O.P.H. No. 11701; 21.10.42.

Origin.—Nelspruit, Transvaal.

State and stage of development.—The plant was in the fresh state and in the flowering stage.

Sheep 62032 (2-tooth; 30·5 Kg.) was given 2·6 Kg. of the fresh leaves and flowers in the course of 9 days.

Result.—Negative.

INSECTS.

Laphygma exempta Walker.

The larvae of this insect are commonly termed kommandowurms or army worms. Reports have repeatedly been received from stock-owners that cattle grazing on pastures heavily infested with the army worm developed gastro-intestinal disturbances (diarrhoea, anorexia). Larvae, which were dead but not decomposed, were minced and given to rabbits as follows:—

Rabbit A (2·1 Kg.) was given 90 gm. of the larvae in one dose.

Symptoms.—Apathy; distended abdomen; dyspnoea; accelerated pulse; general paresis and death in convulsions. The rabbit died 4½ hours after drenching.

Post-mortem appearances.—General cyanosis; severe emphysema and hyperaemia of the lungs; hyperaemia of, and regressive changes in, the liver and kidneys; dilatation of the stomach; slight hyperaemia of the mucous membrane of the stomach and small intestine; tympanites of the caecum and colon.

Rabbit B (1·8 Kg) was given 90 gm. of the larvae in one dose.

Symptoms.—Apathy; dyspnoea; accelerated pulse. The rabbit died 1½ hours after drenching.

Post-mortem appearances.—General cyanosis; hyperaemia, and severe emphysema of the lungs; dilatation of the stomach; slight hyperaemia of the mucous membrane of the stomach; hyperaemia of, and regressive changes in, the liver and kidneys.

Acanthopsyche junodi Heyl.

The pupae of the above insect are termed Wattle bagworms. After removal of the cases (bags), the pupae, some of which were dead, were minced and drenched to a sheep as follows:—

Sheep 53396 (6-tooth; 49·1 Kg.) was given 220 gm. of the pupae in one dose.

Result.—Negative.

The cases of the pupae are spun so that the ingestion of a sufficient number of the pupae will lead to fatal impaction of the rumen in a way similar to that described by Edwards (1935) caused by the ingestion of the pupae of *Gonometa rufobrunnea.*

Mine Compound Waste.

Outbreaks of serious mortality in animals, fed on waste porridge from componds, have relatively frequently been encountered. The evidence in such cases clearly indicates that the waste porridge was the cause of the mortality. Since, however, the animals only take ill some time after consuming the porridge, it is usually impossible to obtain some of the suspected porridge for investigation.

In recent outbreaks of this nature, some of the suspected porridge was, however, obtainable and proved to be toxic by means of biological tests as follows:—

Sheep 53411 (4-tooth; 53·7 Kg.) was given 11·5 Kg. of the porridge in the course of 5 days.

Symptoms.—Severe apathy; salivation; anorexia; general weakness; accelerated, weak pulse; dyspnoea, expiration being accompanied by groaning; severe diarrhoea; stiff gait. The sheep died 5 days after the commencement of dosing.

Post-mortem appearances.—General cyanosis; slight regressive changes in the liver; necrotic pharyngitis; catarrhal and ulcerative abomasitis; hyperaemia of the small intestine; fluid material in the large intestine.

Histology: Liver.—Slight fatty changes.

Kidney.—Slight hyperaemia most marked in the glomeruli.

Sheep 54581 (6-tooth; 46·0 Kg) was given 8·5 Kg. of the porridge in the course of 3 days.

Symptoms.—Apathy; salivation; tympanites; inactivity of the rumen; dyspnoea; stiff gait. The sheep died 4 days after the commencement of drenching.

Post-mortem appearances.—Advanced post-mortem changes; general cyanosis; hyperaemia of the mucous membrane of the small intestine.

Rabbit B (4·0 Kg., was given 180 gm. of the porridge in the course of 4 days.

Symptoms. Anorexia; severe diarrhoea; paralysis of the hindquarters. The animal recovered.

Nails, spent carbide, pieces of leather and decomposed fish and meat are frequently found in the porridge. It is obvious that lamsiekte may be caused by the ingestion of such porridge. All the outbreaks reported followed on or during abnormally hot weather which resulted in very severe fermentation, and even decomposition of the waste porridge, which was left standing in the hot sun.

Summary and Conclusions.

Of the twenty-two plants investigated, six were, for the first time, proved to be toxic, namely *Boscia foetida* Schinz., *Cadaba juncea* (Linn.) B. & H., *Othonna cluytifolia* O.K., *Drimia alta* R. A. Dyer, *Scilla natalensis* Planch, and *Jasminium angulare* Vahl. The results obtained from experiments with *Berkheyopsis echinus* (Less) O. Hoffm. and *B. bechuanensis* S. Moore, *Gliricida maculata* H.B.K. and *Datura arborea* L. are not considered to be conclusive.

The pupae of *Acanthopsyche junodi* Heyl were found to be non-toxic whereas the larvae of *Laphygma exempta* Walker proved to be poisonous.

Waste porridge from compounds was proved to be the etiological agent in an outbreak of poisoning in cattle.

Acknowledgments.

We wish to thank Dr. E. P. Phillips, Chief, Division of Botany and Plant Pathology, Pretoria, and Dr. R. A. Dyer, Senior Botanist in the above Division, for the identification of the plant specimens. To Mr. H. P. A. be Boom, of the Section of Pathology, Onderstepoort, we are indebted for the histological examination of animal organs. Finally, our thanks are due to Messrs. M. G. van Niekerk and P. A. Swanepoel, technical assistants in the Section of Pharmacology and Toxicology, Onderstepoort, for assistance rendered in the course of the experiments.

REFERENCE.

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Fig. 1.—*Boscia foetida* Schinz.

Fig. 2.—*Othonna clutiaefolia* O.K.

Fig. 3.—*Jasminium angulare* Vahl.

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Paresis in Pigs in Relation to Nutritional Deficiencies.

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I. INTRODUCTION.

PARESIS or posterior paralysis of swine is a condition which occurs fairly frequently on pig farms in South Africa. The available literature as well as our own experience shows that it can form part of the symptom complex of a good many etiologically different diseases such as for instance:—

Nutritional deficiencies, e.g., vitamin, minerals, etc.

Acute infectious diseases, e.g., swine fever.

Specific bacterial neuritis and arthritis.

Intoxications, e.g., lead, arsenic, etc.

Epidural abscesses and growths pressing on cord.

Trauma, i.e., fractures of spine or pelvis.

The type of paresis which we set out to study belongs to the first group mentioned above, namely that of nutritional origin.

Usually growing pigs are affected and sometimes whole litters at once. The condition may develop at any time of the year, but frequently makes its appearance towards the end of winter in the summer rainfall areas and the end of summer in the winter rainfall areas. It is particularly prevalent in

the western or drier parts of the Highveld where maize growing can still be carried on and where pigs are kept as a sideline mainly to utilize spoilt grain. The diet here consists in the majority of cases of white maize unsuitable for the trade on account of being mouldy, discoloured, or light and immature through being frostbitten. In addition skim milk and green feed may be given as and if available.

During the periods mentioned, green feed becomes exceedingly scarce or totally absent, as few farmers in those areas are in a position to grow it under irrigation. For the same reasons milk is also usually scarce at the same time.

The observation had already been made that when sufficient green feed was provided such a paresis did not occur in these same areas and under otherwise similar circumstances.

It was desirable, however, to establish the cause of the condition more specifically in the light of recent work on deficient nutrition and also to determine the best and cheapest curative and preventive treatment under conditions peculiar to the areas concerned. Since vitamin A and calcium were the essentials most likely to be deficient in the diet, experiments were planned in the first instance with rations deficient in one or other or both these substances. It was hoped in this way to determine to what extent the symptoms and lesions so produced corresponded to those seen in cases occurring naturally. At the same time control groups receiving adequate rations containing yellow maize, lucerne meal or bone meal, would indicate to what extent these readily procurable feeds could replace green-stuffs as a preventive when this was not available locally.

II. Historical.

Vitamin A.—The conditions under which avitaminosis A manifests itself in pigs, its symptomatology, its response to treatment and the vitamin A requirements of pigs have already been described by various investigators [Hughes *et al.* (1928), (1929); Hostetler and others (1935); Dunlop (1935, 2); Guilbert and co-workers (1937); Hart and Guilbert (1937); and Møllgaard (1938)]. Furthermore, Foot and co-workers (1938) have recently published such an extensive review and excellent discussion of the literature relative to the vitamin A problem in swine husbandry that no attempt is made here to review the literature again.

Calcium.—As far as the writers are aware Bohstedt and co-workers (1926) were the first to describe "posterior paralysis" in pigs as associated with poorly calcified bones, due to a ration low in certain minerals, especially calcium.

The immediate cause of the posterior paralysis was found to be fractured vertebrae in the lumbo-sacral region. According to these authors it appeared as if the poorly ossified lumbar vertebrae were unable to withstand any sudden and severe contraction of the powerful back muscles, such as occurs when slipping on the floor or when frightened. One or other vertebra would collapse under the crushing effect of such a strain resulting in a comminuted fracture with inward bulging and pressure on the spinal cord, thus acting as a nerve block for the rear extremities. Out of 24 animals on this diet, 8 were afflicted with posterior paralysis and, as stated by the authors, several of these after they got into this paralysed condition

dragged their rear extremities extended behind them. Recently Wintrobe (1939) also reported that when an inadequate supply of minerals was given to pigs spontaneous fractures of the long bones and spinal column occurred.

In view of the excellent review by Mitchell and McClure (1937) on the "Mineral Nutrition of Farm Animals", no attempt is made, as in the case of vitamin A, at a comprehensive review of the literature on the calcium requirements of swine, and only work related to the immediate background of the present experiments will be cited later.

III. Experimental. Work.

A. *Management of Pigs.*

As far as possible the animals were kept in individual pens in a central, well ventilated, brick building. The pens measured 6 ft. by 5½ ft. Adjacent to each pen was a runway to which the animals had free access and where they were exposed to an abundance of sunshine. The runway measured 12 ft by 5½ ft. Both the pen and runway were paved with concrete floors. Each unit (pen and runway) was completely separated from the adjacent ones by brick walls so that the faeces and urine could not get into adjoining pens. The animals remained constantly in their quarters except on one day a week when they were driven for about 50 yards to scales for weighing purposes. A wooden board was placed in each pen for the animals to sleep on but as soon as the animals began to show signs of incoordination in their movements, they were supplied with a very poor quality of dry grass for bedding in order to prevent the affected animals from bruising themselves too much.

The pigs were hand-fed once a day in the morning and the mash was offered in the form of a slop. Fresh tap water was at all times available to the animals in the runway.

B. *Experimental Methods.*

1. *The calcium and phosphorus* contents of the rations were determined according to the methods described by Malan and van der Lingen (1931).

2. *The protein* contents were determined by the Kjeldahl method. The protein was calculated from the total nitrogen by the factor 6·25.

3. *The carotene* was determined in the individual constituents of the rations according to the method of Guilbert (1934).

4. *Vitamin A.*—(*a*) The vitamin A determinations in liver were made on unsaponifiable extracts by means of a Lovibond tintometer according to the method of Davis (1933). The results were calculated according to the method of Moore (1930) and the values expressed as Moore Blue Units. (M.B.U.).

(*b*) The vitamin A content in cod liver oil was determined according to the methods of Holmes and others (1937) and the value expressed as in the case of liver.

5. *Determination of Bone Ash.*—Immediately after slaughtering or death of animals the femurs were dissected free from soft tissues and weighed. The bones, after being broken up by means of an iron pestle and mortar, were placed in beakers and dried thoroughly at 105° C. in an electric oven. They were then wrapped in filter paper and extracted with ether in a fat extractor. After removal from the extractor, the bones were pulverized and about 1 gm. samples were placed in crucibles and dried again in an electric oven in order to obtain their dry fat-free weights. The samples were then ashed in an electric furnace in order to obtain the weights of their ash.

6. *Haemoglobin.*—For the determination of haemoglobin the samples of blood were taken from the marginal vein of the ear. The ear was rubbed with clean cotton wool moistened with absolute alcohol, the rubbing being continued until the vein was clearly dilated. The ear was then pricked with a sterilized needle, 20 c.mm. samples taken for analysis and the haemoglobin level determined by the acid haematin method of Newcomer (1919).

C. *First Experiment.—The Production and Prevention of Paresis in Swine on a Straightforward Vitamin A Deficient Ration.*

1. *Type of Pigs used.*—The animals were bred and raised in the piggeries of this Institute. They included Large Whites, Large Blacks, and crosses between large white sows with a large black boar. They were started on experiment shortly after weaning and were from 22 to 28 lb. in weight.* The dams of these piglets received a ration very rich in carotene inasmuch as the mash contained about 65 per cent. of yellow maize. In addition each sow received about 8 to 10 lb. of fresh lucerne per day. After weaning, and until the piglets were put on experiment, they received the same mash as their mothers together with an abundance of green food and it can be assumed, therefore, that by the time they were put on the experiment they should already have stored appreciable amounts of vitamin A.

2. *The Experimental Rations.*—The composition of the rations used is given in Table 1 and the carotene and vitamin A contents of the various ingredients are tabulated in Table 2. From these tables it is clear that all three rations are optimum in proteins, calcium and phosphorus. Rations II and III are also very rich in the carotenoid pigments. When calculated from the values given in Table 2 and, disregarding the carotene figures for meat meal and bone meal, it is found that ration II contained 242 mg. and ration III 440 mg. carotene per 100 lb. of food whereas Dunlop (1935, 2) gives 60 mg. per 100 lb. food for maintenance and normal growth. That is, these rations contained from 4 to 7 times the optimum amount of carotene for normal growth. Ration I, on the other hand, was extremely low in carotene. Even when the highest carotene values are taken for white maize meal, meat meal and bone meal, ration I only contained about 6·1 mg. carotene per 100 lb. food. In other words, a pig weighing 100 lb. and consuming, say, 5 lb. of this ration will only get about 0·3 mg. carotene per day which, according to Guilbert and others (1940) is only one-fourth of the minimum carotene requirement.†

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* The males were castrated about a week before the experiment started. See Table 5 for plan of experiment.

† According to Guilbert and coworkers (1940) the minimum carotene requirement for swine was found to be 25 to 39 micrograms daily per kilogram body weight, that is, 1·14 to 1·77 mg. carotene per 100 lb. body weight.

3. *Results.*

(*a*) *Growth of Pigs.*—The results given in Table 3 show that the pigs* on the high carotene rations gained about three times as much as those on the low carotene diet. The average daily gains were 0·45, 1·48 and 1·35 lb. for Groups I, II and III respectively. Furthermore, Group II (yellow maize) made the best use of their food and Group I the poorest, when expressed on the basis of feed per 100 lb. gain. Group III required 20 lb. more feed than Group II in order to make 100 lb. gain, which was probably due to the greater fibre and lower metabolizable energy content of ration III. Figures 1, 2 and 3 emphasize further the differences in growth and appearance of the various groups. (See Figures 1, 2 and 3.)

(*b*) *Curative Treatment.*—Three pigs were subjected to a curative treatment after symptoms of a vitamin A deficiency had developed. Figure 4 illustrates "Posterior paralysis" in pig No. 42 after it had been fed on ration I for 129 days. At this stage 100 ml. of cod-liver oil daily for 10 days was given and thereafter the animal was killed (Figure 5). "Anterior paralysis" and marked scoliosis were seen in pig No. 30 after 186 days (Figure 6). Figure 7 represents the same animal after it had received cod-liver oil (50 ml. daily for the first 4 days and 25 ml. from then onwards) for 18 days. The scoliosis remained even after 102 days of such treatment (Figure 8).

Although pig No. 53 had been kept on a vitamin A deficient diet for 129 days no clinical symptoms except unthriftiness and retarded growth developed (Figure 9). Thereafter it received 25 ml. cod-liver oil daily for 121 days. Marked improvement occurred as shown in Figure 10.†

Treatment was only started after the symptoms in the two "paralysed" animals were well advanced. For the first few days the oil and some food had to be given in the form of a thin slop by a stomach tube. Two days after the commencement of the treatment the spasms stopped, and after two more days (of treatment) both animals were able to stand up again and support their body on all four legs. The growth curves of two of these pigs before and during treatment are illustrated in Figure 11 and the growth and clinical symptoms of these animals are tabulated in Table 4. (See Figures 4, 5, 6, 7, 8, 9, 10 and 11.)

(*c*) *Vitamin A Content of the Livers.*—The data on the vitamin A content of the livers are set out in Table 5. From the results it is clear that no trace of vitamin A was found in the livers of the pigs receiving only the vitamin A deficient ration (ration I). However, when this ration was supplemented with cod-liver oil, the animals stored considerable amounts of vitamin A in their livers. For instance, the feeding of 100 ml. of cod-liver oil daily for 10 days to a vitamin A depleted animal (Pig No. 42, female, before slaughtering) resulted in a storage of over 67,000 M.B. Units. This amount of vitamin A was about as much as the average amount of vitamin A stored in the livers of pigs on rations II and III where an abundance of carotene was supplied in the forms of yellow maize and lucerne meal respectively.

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* There were 7 pigs in Group I (low carotene) and 3 each in Groups II and III (both high in carotene).

† According to Foot and coworkers (1939) this quantity given daily should be more than sufficient to produce normal growth and health in pigs on a vitamin A deficient basal ration.

D. *Second Experiment.—The Production and Prevention of Paresis in Pigs on a Ration Deficient in Vitamin A and/or Calcium.*

1. *The Pigs Used.*—These animals were also bred at this Institute and consisted of Large Whites and crosses between Large White sows with a Large Black boar. Their management was similar to that of the pigs in the previous experiment. When started on experiment the pigs in Groups IV (lot 1), V, VII and VIII (see Table 3) weighed from 18 to 27 lb. In view of the fact that the pigs in Group IV, lot 1, fared so badly,* the work on this group was repeated with a second lot of animals. In order to allow these pigs more time in which to store vitamin A and calcium, the animals, at the start of the experiment, were older and weighed on the average about 11 lb. more than those in lot 1. As these pigs received white maize and skim milk as ration, it was decided to include also a conformable group on yellow maize and skim milk. These animals constituted Groups IV (lot 2) and VI (Table 3).

2. *The Rations Used and a Discussioin of their Composition.*—In view of the fact that the socalled "posterior paralysis" occurs so often amongst pigs in this country when fed grains and skim milk, it was deemed advisable also to produce the condition in pigs on rations consisting of grain or grain mixtures and skim milk. The composition of the grain mixtures is given in Table I. These mixtures were fed with skim milk in the proportion of 1:1, that is, 1 ml. of milk to every gram of grain. The Ca, P and protein contents of the dry skim milk-grain mixtures are given in Table 6. The carotene content of these rations was calculated from the carotene values of the various ingredients in the rations.

(*a*) *The Proteins.*—The skim milk and grain were fed in the proportion of 1:1, because according to Hart, Steenbock and Letcher (1920) the best utilization of this protein mixture was obtained when fed to pigs in the above proportion. Almost similar and simultaneous results were obtained by Osborne and Mendel (1920) with rats.

Table 6 shows that the protein content of the rations varied from 12 to 13 per cent. According to Mitchell and Hamilton (1935) such levels are too low to produce maximum growth in young pigs. Contrary to the above, based on the results of their experiments, Woodman and co-workers (1939), (1940) concluded that no difference in the growth of pigs kept on rations of 12 per cent. and higher protein content occurred if the growth period was limited to produce a 200 lb. live weight. Only during the earliest stage of the feeding period did the pigs on the low protein diet (12 per cent.) show a slight, though significantly lower rate of live weight increase and poorer efficiency of food conversion than the pigs on standard and high protein diets, "but such differences had ceased to be manifested by the time the pigs had arrived at 60 lb. live weight, and the slight initial disadvantage experienced by the low-protein pigs was wiped out during the later stages of the feeding period". As a matter of fact their results show that the initial setback on the low-protein ration was actually made up again in the period from 150-200 lb. live weight. Therefore, the rations used in the present experiment with only about 12 per cent. proteins of which 28 per cent. is supplied by milk should give good growth in pigs, as was found to be the case, provided the rations are complete in all other respects.

* Three out of four animals fractured their vertebral columns whereas the remaining one made very poor growth and frequently suffered from diarrhoea.

(*b*) *Calcium.*—From Table 6 it is evident that rations IV and VI are very low in calcium, with an average of only 0·13 per cent. Ca in the dry ration. This level is, according to various investigators [Møllgaard (1934), Dunlop (1935, 1), Theiler, du Toit and Malan (1937) and Mitchell and McClure (1937)] by far too low for normal calcification whereas the concentration of calcium in the remaining rations was optimum for growth and bone formation.

(*c*) *Phosphorus.*—According to the publications of Dunlop (1935, 1), Aubel, Hughes and Lienhardt (1936, 1), (1936, 2), Mitchell and co-workers (1937) and Mitchell and McClure (1937) pigs at weaning age require about 0·3 per cent of phosphorus in their ration and it is, therefore, clear that the phosphorus contents in our rations were adequate for growth and calcification.

(*d*) *Iron.*—Ranganathan (1938) gives the iron content of dry maize as 2·3 mg. iron per cent. on an average and that of skimmed milk as 0·24 mg. per 100 ml. Therefore, a pig weighing 25 lb. and consuming 500 g. maize and 500 ml. milk will receive 12·7 mg. Fe and a pig of 150 lb. and consuming at least four times as much food will get 50·8 mg. iron. Although the amount of iron to maintain a positive balance in pigs is not known with certainty, it seems that the above amounts of iron in the food should be adequate to maintain a normal haemoglobin concentration in the blood.*

(*e*) *Copper.*—According to the feed analyses of Elvehjem and co-workers (1929) 100 g. of dry maize contain about 4·5 mg. Cu per Kg. and it seems, therefore, that the copper requirements of pigs should be adequately supplied by a ration consisting of equal parts of maize meal and skim milk.†

(*f*) *Sodium.*—From the figures given by Sherman (1937) a ration consisting of skim milk and maize as fed in this experiment would contain about 0·079 per cent. sodium. This is almost nine times the minimum level fed by Schoorl (1936) and, judging from his results and those of Sinclair (1939), it would seem that for pigs a skim milk and maize ration should not be deficient in sodium.

(*g*) *Chlorine.*—According to Sherman's (1937) compilation a pig weighing 50 lb. and consuming at least 1,000 g. maize and 1,000 ml. milk per day will receive about 1·5 g. of chlorine which, as judged from the results of Woodman and others (1937), seem to be adequate for the normal functioning of the body.

(*h*) *Vitamins: thiamin, pyridoxin, riboflabin and nicotinic acid.*—From the work of Chick and others (1938, 1), (1938, 2) and Hughes (1939) it is evident that, of the vitamin B-complex thiamin (vitamin B_1), pyridoxin (vitamin B_6), riboflavin and nicotinic acid are all essential in the nutrition of the pig.

Judged from the minimum requirements of thiamin and riboflavin for the growing pig as stated by Hughes (1940, 1), 1940, 2) and Van Etten *et al.* (1940), and the vitamin tables compiled by Fixsen and Roscoe (1940),

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* For human beings Sherman (1937)) gives 12 mg. of iron per adult per day as the standard requirement.

† From the copper balance experiments made by Chou and Adolph (1935) the copper requirement of man is approximately 2 mg. per day.

it seems that a ration consisting of equal parts of maize and skim milk should contain adequate amounts of these factors for normal growth. Although the minimum requirement of nicotinic acid for swine has not yet been determined, one is forced to conclude from the work of Chick *et al.* (1938, 2) and Hughes (1939), and from the distribution of nicotinic acid in foods (Bacharach, 1941) that the maize-milk ration, as fed in this experiment, contains enough nicotinic acid for swine. Similarly, the work of Schneider and co-workers (1939) and Swaminathan (1940) lead one to the conclusion that the ration used in this experiment is optimum with respect to vitamin B_6.

(*i*) *Carotene.*—From Table 6 it is clear that the rations of Group VI, VII and VIII contain more than enough carotene for normal growth. Their carotene contents varied from 250 to 408 mg. per 100 lb. of food with an average value of 305 mg. which is more than five times the amount given by Dunlop (1935, 2) as necessary for normal growth. The rations of Groups IV and V only contained about 6·8 mg. carotene per 100 lb. food which is about a ninth of the optimum standard laid down by Dunlop. The carotene contents of rations IV and V are slightly better than that of ration I (Table 1) and it is evident, therefore, that these two rations are also very deficient in the provitamin A factor.

From the above discussion it would seem that the grain-skim milk rations as used in this experiment are optimum for growth except for a few which were low in carotene and/or calcium as indicated in Table 6.

3. *Results.*

(*a*) *Growth of Pigs.*—The results presented in Table 3 show that the pigs on normal rations (Groups VII and VIII) required the smallest amount of feed per 100 lb. of gain, that is, they made the best use of their food. Next in order came the calcium deficient (Group VI), the vitamin A deficient (Group V) and lastly the animals on a calcium and vitamin A deficient ration (Group IV, lots 2 and 1). The results (Group VI) also show that, if young pigs were allowed enough time to store appreciable amounts of calcium in their bone system, they could safely be changed over and fed to market weight on a very economical ration of skim milk and yellow maize with excellent results in growth and well-being. When killed, No. 84, male, weighed 343 lb. after having been on skim milk and yellow maize for 196 days. When the grain is white maize (Group IV, lot 2), retardation in the growth of pigs sets in presumably as soon as their vitamin A reserves are exhausted. The differences in growth between the various groups are further shown in Figures 12, 13, 14, 15, 16 and 17.

(*b*) *Haemoglobin Level in Blood.*—The results of these haemoglobin determinations are given in Table 7. From these figures it is seen that the great majority of them fall between 10-12 grams haemoglobin per 100 ml. of blood. This range is somewhat higher than the "normal" haemoglobin value for pigs (8-10 grams per 100 ml. of blood) as found by Hart and co-workers (1930) but it is in close agreement with the normal values of Hamilton and associates (1930) and Chick and co-workers (1938, 2) for young pigs. The results also support the work of Hart and associates (1930) in so far that a ration of skim milk and maize is optimum for haemoglobin synthesis in young pigs.

(*c*) *Curative Treatment for Vitamin A Deficiency.*—Four pigs, Nos. 81, 83, 85 and 48, after they showed symptoms of vitamin A deficiency, were subjected to a curative treatment with cod liver oil. Table 4 and figures 18, 19, 20 and 21 show the growth and condition of the animals before and after the curative treatment.

(*d*) *Weight of, and Percentage Ash in Femurs.*—Some of the bones analysed were from pigs that had died rather early in the experiment, or from animals that showed very poor growth. These facts, no doubt, weaken the significance of the values presented in Table 8, especially those of the fresh weight of the femurs for the various groups. Nevertheless, the ash in the femurs of pigs whose rations were supplemented with bone meal, seem to be significantly higher than the ash in femurs of pigs on the low calcium rations. For instance, the percentage of ash in the dry fat-free femurs of the pigs receiving bone meal (Groups V, VII and VIII) was on the average 61·60 per cent. whereas the average percentage of ash in the femurs of pigs fed the low calcium rations (Groups IV and VI) was 55·69, that is, a difference of 5·91 per cent. ash in favour of the bone meal fed animals.

(*e*) *Vitamin A Content of the Livers.*—The data concerning vitamin A content of the livers are tabulated in Table 5. As in the previous experiment the results indicate that there was no trace of vitamin A in the livers of pigs fed the vitamin A deficient rations (Rations IV and V), whereas when these rations were supplemented with cod-liver oil, the animals stored appreciable amounts of vitamin A in their livers. Likewise, the livers of animals fed yellow maize or lucerne meal (Rations VI, VII and VIII) contained large amounts of this vitamin.

IV. Clinical Symptoms.

Groups I and V ($-A+Ca$) (*Ration Deficient in Vitamin A*).

The first symptoms appeared from about the 50th day of low vitamin A diet. Our experience that the symptoms vary in different animals coincides with that of other observers. The symptoms enumerated below did not, therefore, necessarily occur in every affected animal.

Usually the first sign observed in pigs on this diet is a falling off of the appetite with consequent retardation of growth noticeable already from the 40th day. The animals appear less thrifty and progressively the skin becomes dry and scaly, the hair or bristles shaggy and dull, soiled and eventually split at their tips. The conjunctiva becomes reddened and a thin sero-mucous secretion running down from the medial canthus tends to soil the skin along its path. Later it becomes more viscid and stains the skin light brown. Gradually the animal's vision becomes affected, the pupils remain in a state of continuous dilation, the eyeball seems to protrude giving rise to a peculiar staring effect, probably on account of increased intraocular pressure. The iris reacts to weak light very slightly or not at all but contracts slowly under influence of bright sunlight.

Excepting one pig (No. 55) which had a slight corneal opacity and ulcer no macroscopic changes could be seen externally to explain the gradual loss of sight. Erosions and bruises of the snout, nostrils and other parts of the skin of the limbs evidently resulted from animals walking into obstacles. Later the animals seemed to become more wary and were able to avoid this, while still able to find their way about to the food trough and return to

their stye. After some time the ears began to droop and in a few animals a ventral or lateral curvature of the spine (lordosis, scoliosis) developed. This was usually accompanied by a twisting of the head to one side (torticollis).

The animals either remain quiet and even lethargic or become restless and irritable, moving about continuously and aimlessly in circles.

The limbs usually become very straight and the gait then becomes awkward, stiff or stilted and short stepped. In other animals owing to swaying of hindquarters and knuckling over at the fetlocks the gait was even more uncertain. The incoordination of movements becomes progressively more severe and leads ultimately to paresis and even paralysis of the hindquarters so that the animal can only raise its body with its fore limbs to assume a sitting posture. Eventually the animal is unable to do this even with assistance and may develop skin abrasions from unsuccessful attempts to rise. In one case (No. 30) paralysis of the forequarters preceded that of the hindquarters, the body being supported on the knees at first. In addition this animal had difficulty in eating and swallowing its food. It would take up some food into its mouth and then raising its head gulp it down with an exaggerated snapping movements of the jaws.

From about the 130th day a nervous collapse occurred in most animals. This was frequently heralded by convulsive fits which were brought about fairly easily when the animal was roused and urged to rise first thing in the morning. When this had passed off, another fit or two could be brought about less easily by exciting the animal. For instance, a fit might be induced by spraying the animal with some insecticidal solution against lice. The animal would be heard to squeal and become agitated. Then it would sag on its haunches with a peculiar stare, and fall on its side. The legs made rapid galloping movements and were later extended stiffly. The head was thrown well back and the respiration became laboured and reduced in number. Finally the animal became exhausted and lay gasping for a minute or two. After several unsuccessful attempts at rising the animal could eventually assume a sitting posture and even get on to its feet and walk with a very uncertain wobbly gait to its trough and start feeding.

These pigs showed a certain tendency to allotriophagia. They were often observed gnawing at the bricks and mortar of their stye and whenever taken out invariably started eating earth and sand. Some of the pigs developed diarrhoea at intervals.

Gilts came on heat at irregular intervals and seemed to remain in this state for unusually long periods.

One pig (No. 52) died after showing surprisingly few symptoms apart from gradual loss of appetite. One day it was found lying down and unable to rise. No pain or other sign of severe illness could be detected and its death a few hours later was quite unexpected. This is in accordance with the work of Foot and others (1938). Observations made on animals after treatment with cod liver oil will be found in Table 4.

Group IV, Lots 1 *and* 2 (- *A* - *Ca*).—The early and marked retardation of growth in Lot 1 of this group is strikingly illustrated in Figure 12. The earlier symptoms are similar to those of the previous group, except that convulsive fits and impaired vision were not noted. There was the same watering of the eyes with soiling of the skin from the medial canthi down. The gait was similar with short, stiff steps, the limbs being very

straight as if the animal was attempting to tiptoe. Before symptoms could develop further, 3 out of the 4 pigs became suddenly and completely paralysed in the hindquarters due to fracture of a lumbar or posterior thoracic vertebra. The remaining animal had to be killed owing to a severe arthritis of a hind limb.

Group VI ($+A-Ca$). (*Ration Deficient in Calcium*).—Except for pig No. 80 which developed into a runt after weaning and consequently made very poor growth, the remaining three pigs in this group grew well, making excellent gains in weight and showed no abnormal symptoms. (See Figures 22 to 25.)

Groups II and VII ($+A+Ca$).—Carotene was supplied by yellow maize. A painful swelling involving both carpal joints occurred in pig No. 58 after some considerable time. This was manifested by the animal first walking with a stiff gait in the forelimbs and later, as the condition became worse, it lay down and resented to rise even when urged to get up. A carpitis was diagnosed. The remaining 6 pigs in these groups developed well and remained healthy until the end of the experiment.

Groups III and VIII ($+A+Ca$).—Carotene was supplied by lucerne meal. The seven pigs in these groups developed well and remained in a good state of health during the experiment.

V. The Anatomical Pathological Changes.

Groups I } $-A+Ca$ { *White maize, meatmeal and bonemeal.*
V } { *White maize, skim milk and bonemeal.*

Of the eleven animals which were kept on this ration four pigs were killed after they had been treated for periods varying from 10 to 140 days. Of the remaining seven, four died as a result of affections of the lungs after they had been in the experiment for periods varying from 99 to 317 days.

We found that not only were the post-mortem findings of the treated animals different from those of the untreated ones, but they even varied from animal to animal in the latter group.

(*a*) *The Post-mortem Findings in the Untreated Animals.*—The following changes were common to all the pigs in this group. The carcass showed signs of retardation of growth. A dry brownish secretion soiled the skin below the medial canthi of both eyes. The hair coat was lustreless and the bristles had a tendency to split at their tips. The surface of the skin was drier than usual and was covered with scabs especially over the back. Abrasions involving the skin over the orbits, the elbow and hock joints, the fetlocks and above the coronet appeared, however, in some of the animals (Pigs Nos. 27, 39, 43 and 55). Small red spots, which tend to form pustules later occurred in different parts of the skin (pig No. 44) and raised papules were distributed over the body (pig No. 42).

General cyanosis of the visible mucous membranes and of the unpigmented skin especially of the abdomen was present in the animals suffering from pulmonary affections (pigs Nos. 27, 39, 43 and 52). In others (Nos. 29, 44 and 55) the conjunctiva was reddish discoloured and the blood-vessels were congested.

On opening the carcass a slight ascites, hydrothorax and hydropericard was found in all the animals.

A fibrinous pleuritis and a necrotic pneumonia (pig No. 27), fibrous adhesions of the left lung to the diaphragm and localized bronchitis (pig No. 29), congestion of lungs and aspirated ingesta in the larynx and trachea (pig No. 39), congestion and oedema of the lungs (pig No. 52), a localized chronic pleuritis involving an area over the 4th to the 6th rib, a purulent pneumonia and an aspirated oily material in the bronchi and trachea (pig No. 43) occurred in the animals with respiratory affections.

In 5 animals the gastro-intestinal mucous membrane was slightly swollen and hyperaemic (pigs Nos. 27, 39, 44, 52 and 55) and a slight ascaris infestation was present in 3 pigs (Nos. 55, 29, and 43).

Fatty changes of the kidneys, a hydronephrosis of the left kidney, distension of the pelvis and the ureter and a posthitis were observed in pig No. 29.

In pig No. 55 a small corneal ulcer occurred on the right eye and a slight opacity blurred the cornea of the left. Calluses involved the distal end of some ribs and on palpitation, these could easily be mistaken for enlarged costochondral junctions.

(*b*) *The Post-mortem Findings in the Treated Animals.*—On the whole the condition of the treated pigs was much better than that of the untreated ones. The secretion soiling the medial canthi was reduced (pig No. 42) or totally absent (pigs Nos. 30, 42 and 53). The hairy coat had improved, the bristles had become more dense and had more lustre than those of the untreated pigs. The skin on the whole had also improved. It was more pliable and less scabby than in the former lot. Raised papules were distributed over various parts of the body, and in addition marked chronic pleuritis, localized pneumonic changes in both lungs and hydronephrosis of the left kidney were seen in pig No. 42.

Except for a fairly pronounced ascaris infestation, no abnormal changes were noted in pig No. 48.

Group IV (1) *and* (2) (– *A* – *Ca*) *White Maize Meal and Skim Milk in Proportion of* 1 : 1.

Of the seven animals kept on this ration for periods varying from 69 to 290 days, three (Nos. 81, 83 and 85) were treated with cod liver oil for from 12 to 42 days and the remaining four received no treatment. Of the latter animals three suffered from a fractured spine. Of the former one animal (No. 81) died after faulty drenching and the remaining two were killed at the end of the experiment.

(1) *Post-mortem Findings in the Untreated Animals.*—Similar changes to those described for the untreated animals in Groups I and V were seen in the four pigs (Nos. 41, 50, 54 and 57) kept on this ration. The signs of stunted growth and cachexia, however, were more pronounced, and the marked softening of the skeleton was of interest. Evidence of it was reflected in the callus formation on several ribs on both sides of the thorax. In three animals (Nos. 41, 54 and 57), there was fracture of the spine at the second last thoracic vertebra, the fourth lumbar vertebra and the third lumbar vertebra. The consistence of the ribs was markedly reduced, they bent like cardboard and broke without a snap. A peri-arthritis and arthritis occurred in the left tarsus of pig No. 50.

In these animals, as the result of struggling before death, excoriation of the skin occurred in various places. The point of the medial claw of both fore limbs was worn through (pig No. 41) and bleeding occurred. Retention of urine occurred in one pig (No. 41). Several pigs were affected with some lice (*Haematopinus suis*).

Signs of diarrhoea, colitis and a slight ascaris infestation were seen in pig No. 50.

Marked haemorrhages occurred in the neighbourhood of the fractured vertebra and the blood infiltrated into the adjacent portion of the psoas muscles.

The prepuce was swollen and catarrhal changes were present (pig No. 41).

(2) *Post-mortem Findings in the Treated Animals.*—On the whole the condition of these animals was better than that of the untreated lot. Nothing unusual was noticed except multiple calluses involving several ribs on both sides of the thorax. The consistence of the ribs increased proportionally to the period of treatment. The calluses are very distinct, bulging deeply into the thoracic cavity and the consistence of the ribs was either soft or brittle. A varying infestation of ascaris was present, which was fairly heavy in pig No. 81. In addition cyanosis of the visible mucous membranes, deformity of the claws with ulceration below and a necrotic stomatitis occurred in pig No. 81.

Group VI (+A −Ca) Yellow Maize.—The four animals kept on this ration were killed at the end of the experiment. They had received no cod liver oil. On autopsy nothing unusual was observed, except that the consistence of the ribs was slightly reduced. The ribs broke with a snap on being bent and pressure applied, but in one case (pig No. 80) the lower portion of the ribs bent like cardboard. Incidentally this pig had been kept the shortest period on the diet and it was on the whole unthrifty.

A few ascaris were found in the intestine of all the animals, and there were haemorrhages in the thoracic cavity.

Groups II and VII (+A +Ca) Carotene supplied by Yellow Maize.—The autopsy on seven pigs kept on this ration revealed nothing unusual, except a slight ascaris infestation. Several pigs were infested with lice (*Haematopinus suis*).

A carpitis sicca involving both fore-limbs was diagnosed in one animal (pig No. 58).

Groups III and VIII (+A +Ca) Carotene supplied by Lucerne Meal.—The seven pigs were killed at the conclusion of the experiment. The results of the autopsy were similar to those of the former two groups, except that no carpitis was diagnosed.

Considerable material has been collected from these experiments for histological study, which unfortunately could not be completed in time This part of the work is intended for later publication.

VI. Conclusions.

(1) Evidence so far accumulated seems to show that under veld conditions in South Africa, a vitamin A deficiency occurs fairly frequently amongst pigs. The following are the conditions that are probably chiefly responsible for the occurrence of this type of malnutrition in this country.

(a) *Climatic.*—In the winter rainfall areas the dry season usually extends from October to March and in the summer rainfall areas from March to October or even later. On most farms during these periods, lasting 6 to 7 months, little or no green food is available. Based on the evidence of our experiments it would seem that such a period of low vitamin A intake is long enough to manifest its deleterious effects, particularly in the young of fast-growing species such as the pig. The appearance of symptoms and degree of affection will depend naturally on the amount of vitamin A previously stored in the animal's body.

(b) *Feeding.*—When supplementary feeding is practised, the rations in the winter rainfall areas are usually composed of barley or rye meal plus one or more of the following ingredients: wheaten bran, minerals, meat meal, peanut meal and skim milk. In the summer rainfall areas the same ingredients are incorporated in the rations except that maize is usually substituted for barley or rye. Unfortunately, on account of the better market, white maize is planted mainly in the summer rainfall areas and it constitutes the staple concentrated stock feed in these areas. It is also well known that the feeds listed above are all deficient in the carotenoid pigments and are, therefore, unable to supply the vitamin A requirement.

(2) In order to rectify the adverse feeding conditions during the dry seasons in this country attention should not only be given to the probable energy, protein and mineral deficiencies in the pasture but also to the vitamin A requirements of the grazing stock.

(3) The most practical and probably also the most economical way of supplying the essential carotene (vitamin A) to the animals during the dry seasons seems to be in the form of yellow maize and lucerne meal (hay). Considering the variable carotene content in different samples of these products and allowing for a margin of safety, it seems that 30 to 50 per cent. of yellow maize or 5 to 8 per cent. of lucerne meal in the ration should supply enough carotene to tide the animals over the adverse seasons.

However, the recommended levels of maize in the ration of pigs may have an undesirable effect on the quality of the fat. Therefore, it may be advisable for those farmers who go in for the production of baconers, further to reduce the proportion of maize in their rations and to include also some lucerne meal or 0·5 per cent. cod liver oil as recommended by Foot and associates (1939).

(4) A rather common fattening ration for pigs in this country consists of a cereal meal with skim milk. If the cereal is yellow maize and if it is fed with skim milk in equal amounts by weight, the ration will be complete with respect to growth except that it is low in calcium. This can be rectified by the addition of either 1·5 per cent of fluorine-low agricultural lime or 2 per cent. of bone meal to the maize meal. To improve the palatability 1 per cent. of common salt may also be added. If the cereal is white maize or if it belongs to the barley, rye, etc., group, provision should not only be made for the calcium but also for the necessary carotene or vitamin A in the cheapest and most readily form available, e.g., green stuff, pumpkin, lucerne meal or even cod liver oil.

VII. Summary.

1. A form of paralysis or paresis prevalent in some parts of the Union in young pigs is described. There was evidence already that it was due to a deficient diet.

2. Experiments were carried out to establish the cause of this disease more definitely by feeding to young pigs rations low in vitamin A, in calcium or both.

3. The pigs deficient in vitamin A developed symptoms which in the earlier stages at any rate correspond with those seen in the natural disease. An account of the symptoms and pathological changes noted in this artificial avitaminosis is given.

4. The pigs on a combined vitamin A and Ca deficient diet developed such a softening of the skeleton that within 80 days three out of four fractured their spine and had to be destroyed. Other pigs started on the same ration when somewhat older, and which presumably, therefore, had a greater calcium (+ vitamin A)reserve in their body, did not develop such extreme lesions.

5. Gilts on a vitamin A deficient diet showed irregularity in the oestrous cycle. Oestrus occurred more frequently and persisted for abnormally long periods.

6. A ration of equal parts by weight of skim milk and white maize is physiologically complete for growth in pigs except that it is low in calcium and in vitamin A.

The incorporation of 2 per cent. bonemeal and 10 per cent. lucerne meal in such a diet or the substitution of yellow maize and bone meal for white maize resulted in normal growth and good health in pigs. Where green feed or other cheap sources of vitamin A are not available in adequate quantities, such a supplementation should prevent all tendency to paralysis and poor growth.

7. Cod liver oil administered to animals even in advanced stages of the deficiency effected rapid and striking improvement in health. Some of the lesions, however, could not be cured completely, e.g., bad cases of scoliosis and blindness.

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APPENDIX.

TABLE 1.

Composition of Rations in Percentage by Weight.

Group No.	I. − A + Ca.	II. + A + Ca.	III. + A + Ca.
Type of Ration.*	Carotene-poor (White Maize Meal).	Carotene-rich (Yellow Maize Meal).	Carotene-rich (Lucerne Meal).
White maize meal	86	—	77
Yellow maize meal	—	86	—
Meat meal (safco)	10	10	9
Lucerne meal	—	—	10
Bone meal	3	3	3
Sodium chloride	1	1	1
	100	100	100
Proteins .... Per cent.	15·70	16·17	16·08
Ca .... ,,	0·70	0·77	0·89
P .... ,,	0·52	0·51	0·57
Ca : P ratio	1·35 : 1	1·51 : 1	1·56 : 1

TABLE 1 (*contiued*).

Composition of Grain Mixtures.

Group No.	IV.	V.	VI.	VII.	VIII.
White maize meal	100	97	—	—	87
Yellow maize meal	—	—	100	97	—
Lucerne meal	—	—	—	—	10
Bone meal	—	2	—	2	2
Sodium chloride	—	1	—	1	1
	100	100	100	100	100

* The signs (−) = deficient; (+) = optimum; and (A) = provitamin A.

TABLE 2.

The Carotene and Vitamin A Contents of Various Feeds.

Feed.	Weight of Sample (on Air Dry Basis).	Carotene Content (on Air Dry Basis).	Vitamin A. Content.
	g.	Mg. Per cent.	
Yellow maize meal	100	0·62	—
White maize meal	100	<0·01	—
Lucerne meal	30	9·70	—
Meat meal (safco)	100	<0·04	—
Bone meal	200	<0·03	—
Skim milk (Willstaedt and With, 1938)	100 ml.	0·005 + ≡ 0·007	3 I.U.*
Cod liver oil	0·037 g.	—	535 M.B.U. per gram or 495 M.B.U. per ml.

* One I.U. ≡ 0·6 microgram of pure beta carotene (Daniel and Munsell (1937).]
< Means less than.

TABLE 3.

Gains and Feed Consumption of Pigs on Rations Low and High in Carotene Content. (Period 129 days. All weights expressed in pounds.)

	GROUP I. (Low Carotene).	GROUP II. (High Carotene).	GROUP III. (High Carotene).
Source of carotene	—	Yellow maize	Lucerne meal
Average initial weight	24·0	22·3	22·0
Average final weight	82·5	214·0	195·7
Average gain	58·5	191·7	173·7
Average total food	241·0	555·0	539·0
Average daily gain	0·45	1·48	1·35
Average daily feed per pig	1·87	4·30	4·17
Average feed per 100 lb. gain	411·0	289·0	310·0

TABLE 3 (*continued*).

Gains and Feed Consumption of Pigs Fed Rations containing various levels of Carotene and Calcium. (Period 147 days. All weights expressed in pounds.)

Type of Ration.*	Group IV.		Group V.	Group VI.	Group VII.	Group VIII
	Lot 1. −A− Ca.	Lot 2. −A−Ca.	−A+Ca.	+A−Ca.	+A+Ca.	+A+Ca.
Source of Carotene	—	—	—	Yellow maize	Yellow maize	Lucerne meal
Average initial weight	22·5	33·0	21·2	32·3	22·0	18·2
Average final weight	51·0	152·0	76·7	192·3	160·3	173·0
Average gain	28·5	118·7	55·5	160·0	138·3	154·8
Average total food	182·0	475·0	215·0	557·0	420·0	485·0
Average daily gain	0·19	0·81	0·38	1·09	0·94	1·05
Average daily feed per pig	1·24	3·23	1·46	3·79	2·86	3·30
Average feed per 100 lb. gain†	638·0	400·0	387·0	348·0	304·0	313·0

* The signs (−) = deficient; (+) = optimum; and (A) = provitamin A.

† Daily feed and feed per 100 pounds of gain on basis of milk being reduced to a moisture content of 4 per cent., that is, 100 ml. of skim milk ≡ 10 g. skim milk powder containing 4 per cent. of moisture.

TABLE 4.

Curative Treatment with Cod Liver Oil.

No. of pig and sex.	Symptoms prior to feeding of cod liver oil.	Growth and food consumption immediately preceeding feeding of cod liver oil lb. per day for 10 week period.		Cod liver oil fed.	Growth and food consumption immediately following commencement of feeding cod liver oil., lb. per day for 10 week period.		Effect of feeding cod liver oil on the symptoms.
		Growth.	Feed intake		Growth.	Feed intake	
42 Female..	The pig was growing fairly up to the time when it went down with "posterior paralysis" after having been on experiment for 129 days. From 56th day on experiment this pig was in heat for an unusually long time. At this period it also became very restless and nervous. Its vision became impaired and the dilated pupils did not respond to strong light. First spasms noticed on 105th day. From 129th day shoulders and sides were full of red papules. Pig collapsed when weighed on 129th day	—	—	100 ml. of cod liver oil daily for 10 days when animal was killed	—	—	Two days after the commencement of cod liver oil treatment the spasms stopped and after two more days of treatment the animal was able to stand up on all four legs. When killed on the 10th day of treatment the gait was still unsteady with hindquarters swaying.
53 Female..	This pig was growing very poorly but had shown no other definite symptoms. It was thin and leggy	0·49	1·71	25 ml. daily from 129 to 269 days on experiment	1·1	3·93	Made good progress and continued in excellent health. Pupils responded normally to light.
30 Male....	This animal grew very poorly and after it had been on experiment for 109 days, it started to show impaired vision, incoordination and spasms. Shortly afterwards the pig began to turn in circles, with its head held on one side. It also had difficulty in eating as it slopped the wet mash up in dog fashion. On the 186th day the animal showed severe scoliosis and spasms. It was prostrated with partial paralysis of its forequarters	0·22	2·04	50 ml. daily from 187 to 190 days and 25 ml. daily from 191 to 289 days on experiment	0·67	3·56	Two days after commencement of liver oil treatment the spasms stopped but it took the animal 12 days before it could walk again. After the animal had received cod liver oil for a week, it broke out in prominent red papules all over its neck and sides. After three more weeks the pustules had dried up leaving the skin hard and scaly. This condition together with the scoliosis and impaired vision lasted right up to the end of three months' treatment.

TABLE 4 (*continued*).

No. of pig and sex.	Symptoms prior to feeding of cod liver oil.	Growth and food consumption immediately preceeding feeding of cod liver oil lb. per day for 6 week period.		Cod liver oil fed.	Growth and food consumption immediately following commencement of feeding cod liver oil., lb. per day for 6 week period.		Effect of feeding cod liver oil on the symptoms.
		Growth.	Feed intake		Growth.	Feed intake	
83 Male.... (Group IV)	In spite of the fact that this pig started to show watering eyes and impaired vision from the 5th month on experiment, it made good growth for the first 7½ months on experiment. From then on its gait became incoordinated and its sides full of red papules. After 9 months on experiment the animal showed severe spasms and partial "posterior paralysis"	0·57	4·91	75 ml. daily from 291 to 293 days on experiment. 50 ml. daily for the next three days and 25 ml. daily for the remaining 36 days	1·62	6·35	The animal made a quick recovery from its partial "posterior paralysis" and gained much in weight during the period of cod liver oil treatment.
85 Male.... (Group IV)	This animal made excellent growth for the first 6 months on experiment. From the 217th day its gait became incoordinated and from the 235th day it showed a severe scoliosis	1·24	7·20	75 ml. daily from 291 to 293 days on experiment 50 ml. daily for the next three days and 25 ml. daily for the remaining 36 days	2·07	8·29	The animal made a considerable increase in weight during the curative treatment, and after 42 days of treatment the scoliosis had practically disappeared.
48 Female (Group V)	This animal, shortly after being put on experiment, developed into a runt. Its appetite was very poor and it subsequently gained very little in weight	*For 10 week period.* 0·12 lb. (per day)	—	25 ml. cod liver oil daily from 117 to 227 days on experiment	*For 10 week period.* 0·57 lb. (per day)	—	The animal made a gradual recovery and eventually made good progress.
81 Female.. (Group IV, Lot 2)	From the 5th month this animal started to show watering eyes but made good gains until end of 7th month. From the 217th day animal became very nervous and showed continuous oestrus for more than a month. On 257th day ulcers were noticed below claws of the right fore leg. From 278th day when animal became paralysed in hindquarters it was dosed with cod liver oil.	—	—	50 ml. cod liver oil daily from 278 to 289 days on experiment	—	—	Unfortunately due to faulty drenching the animal died on 290th day from gangrenous pneumonia

Table 5.

Vitamin A Reserves in Livers of Experimental Pigs.

Group No.	Pig No. and sex.	Type of Ration.*	Days of ration (died or killed).	Weight of pig at termination of life.	Weight of liver (g).	Vitamin A. M.B.U. per gram of liver.	Vitamin A. M.B.U. in whole liver.
				lb.			
I	29 Male....	Deficient in carotene (or vitamin A) —A+Ca	129 (killed)................	102	682	0	0
	44 Male....		129 (killed)................	182	1,230	0	0
	27 Female..		111 (died)................	98	575	0	0
	52 Female..		99 (died)................	53	497	0	0
	42 Female..		139 (killed)................	108	1,055	63·8	67,309†
	30 Male....		289 (killed)................	147	985	436·8	430,248†
	53 Female..		269 (killed)................	257	1,338	586·0	784,068†
II	31 Male....	Normal +A+Ca (carotene supplied by yellow maize	146 (killed)................	187	1,376	67·2	92,467
	33 Male....		140 (killed)................	226	1,590	64·6	102,714
	34 Female..		134 (killed)................	262	1,825	25·7	46,902
III	32 Male....	Normal +A+Ca (Carotene supplied by lucerne meal)	146 (killed)................	226	1,865	28·2	52,593
	35 Male....		140 (killed)................	206	1,512	25·2	38,102
	36 Female..		134 (killed)................	207	1,684	22·4	37,722
IV	54 Male....	—A—Ca..................	69 (died from fractured spinal column)	67	—	—	—
Lot 1	41 Male....		74 (killed—fractured spinal column)	62	592	0	0
	57 Female..		111 (killed—fractured spinal column)	—	485	0	0
	50 Female..		161 (killed)................	50	417	0	0
Lot 2	81 Female..	—	290 (died gangrenous pneumonia)	185	1,866	4·6	8,584‡
	83 Male....		332 (killed)................	354	1,467	67·2	98,582‡
	85 Male....		332 (killed)................	404	2,066	30·8	63,633†

Notes on next page.

TABLE 5 (*continued*).

Group No.	Pig No. and sex.	Type of Ration.*	Days of ration (died or killed).	Weight of pig at termination of life.	Weight of liver (*g*).	Vitamin A.	
						M.B.U. per gram of liver.	M.B.U. in whole liver.
				lb.			
V	39 Male	−A+Ca	202 (died)	70	802	0	0
	43 Male		317 (died)	131	1,408	0	0
	55 Female		252 (killed)	143	801	0	0
	48 Female		237 (killed)	155	1,044	588	613,872‡
VI	80 Male	+A−Ca (yellow maize)	150 (killed)	88	622	50·4	31,349
	84 Male		196 (killed)	343	2,142	95·7	204,989
	82 Female		196 (killed)	280	2,246	73·8	165,755
	61 Female		200 (killed)	204	1,145	123·0	140,835
VII	37 Male	+A+Ca (yellow maize)	147 (killed)	183	1,179	73·9	87,128
	58 Male		140 (killed)	168	1,414	24·6	34,784
	45 Female		158 (killed)	137	895	78·4	70,168
	40 Female		158 (killed)	123	1,006	46·2	46,477
VIII	38 Male	+A+Ca (lucerne meal)	151 (killed)	195	1,481	32·8	48,577
	59 Female		151 (killed)	170	1,267	29·8	37,757
	88 Female		129 (killed)	274	1,945	80·6	156,767
	86 Female		129 (killed)	274	2,226	79·8	177,635

* The signs (−) = deficient; (+) = optimum; and (A) = provitamin A.

† Pig No. 42 received cod liver oil for the last 10 days, pig No. 30 for the last 101 days and pig No. 53 for the last 110 days on experiment (See also Table 4).

‡ These animals, after they started to show symptoms of vitamin A deficiency, were fed cod liver oil for various periods of time (for particulars see Table 4).

TABLE 6.

*Protein, Mineral and Carotene Contents of Dry Skim Milk-Grain Mixtures.**

Group No.	IV.	V.	VI.	VII.	VIII.
Proteins.....................Per cent.	12·0	12·3	12·4	12·5	13·0
Calcium..................... ,,	0·13	0·66	0·12	0·66	0·73
Phosphorus.................. ,,	0·29	0·40	0·29	0·40	0·41
Ca : P ratio........................	·45 : 1	1·65 : 1	·45 : 1	1·65 : 1	1·78 : 1
Carotene, mg................Per cent.	0·015	0·016	0·572	0·555	0·900
Outstanding type of ration†..........	−A −Ca	−A+Ca	+A−Ca (Yellow maize)	+A+Ca (Yellow maize)	+A+Ca (Lucerne maize)

* The skim milk and grain were fed in the proportion of 1 : 1, that is, 1 ml. of milk to every gram of grain. Skim milk contained 9·64 g. solids per 100 ml. of milk when dried at 100° C. The protein, calcium and phosphorus percentages of the rations were made on mixtures consisting of 100 g. air dry grain mixture plus 10 g. of air dry commercial skim milk powder containing 4 per cent. of moisture.

† The signs (−) = deficient ; (+) optimum ; and (A) = provitamin A.

TABLE 7.

Grams Haemoglobin per 100 *ml. of Blood.*

Group Number.	Number of Animals Considered.	Days on Ration.					
		6.	47.	70.	105.	145.	Average.
IV	6*	12·41	11·70	12·69	12·10	11·42	12·06
V	3	13·83	11·69	12·09	10·03	10·59	11·65
VI	3	13·77	11·13	11·96	11·38	14·08	12·46
VII	4	13·89	11·55	12·42	10·51	12·84	12·24
VIII	4	13·33	12·18	13·36	14·23	13·08	13·23

* After the 70th day the haemoglobin figures of Group IV are averages of the values of three animals only.

TABLE 8.

Weight and Percentage Ash in Femurs of Young Pigs Fed Skim Milk and Maize Rations.

Group No.	Pig Number and Sex.	Type of Ration.*	Fresh Weight of Femurs.	Percentage of Ash in Dry Fat-free Femurs.
			Grams.	
IV	54 Male	A Ca	—	54·22
	41 Male		—	52·39
Lot 1	57 Female		139·0	53·76
	50 Female		105·0	55·26
	81 Female		240·0	62·94
Lot 2	83 Male		298·0	59·44
	85 Male		343·5	59·62
		AVERAGE	225·1	56·80
V	39 Male	A – Ca	157·0	61·44
	43 Male		234·5	60·78
	55 Female		2[illegible]·5	64·27
	48 Female		191·0	58·73
		AVERAGE	205·7	61·30
VI	80 Male	A Ca (yellow maize)	112·5	50·86
	84 Male		222·5	54·23
	82 Female		210·5	57·98
	61 Female		201·0	55·29
		AVERAGE	186·6	54·59
VII	37 Male	A Ca (yellow maize)	199·0	62·26
	58 Male		209·5	62·80
	45 Female		181·5	60·73
	40 Female		175·5	63·90
		AVERAGE	191·4	62·42
VIII	38 Male	A – Ca (lucerne meal)	228·0	60·26
	59 Female		216·0	62·03
	88 Female		264·5	60·56
	86 Female		258·5	61·55
		AVERAGE	241·7	61·40

* The signs (–) = deficient; ( ) = optimum; and (A) provitamin A.

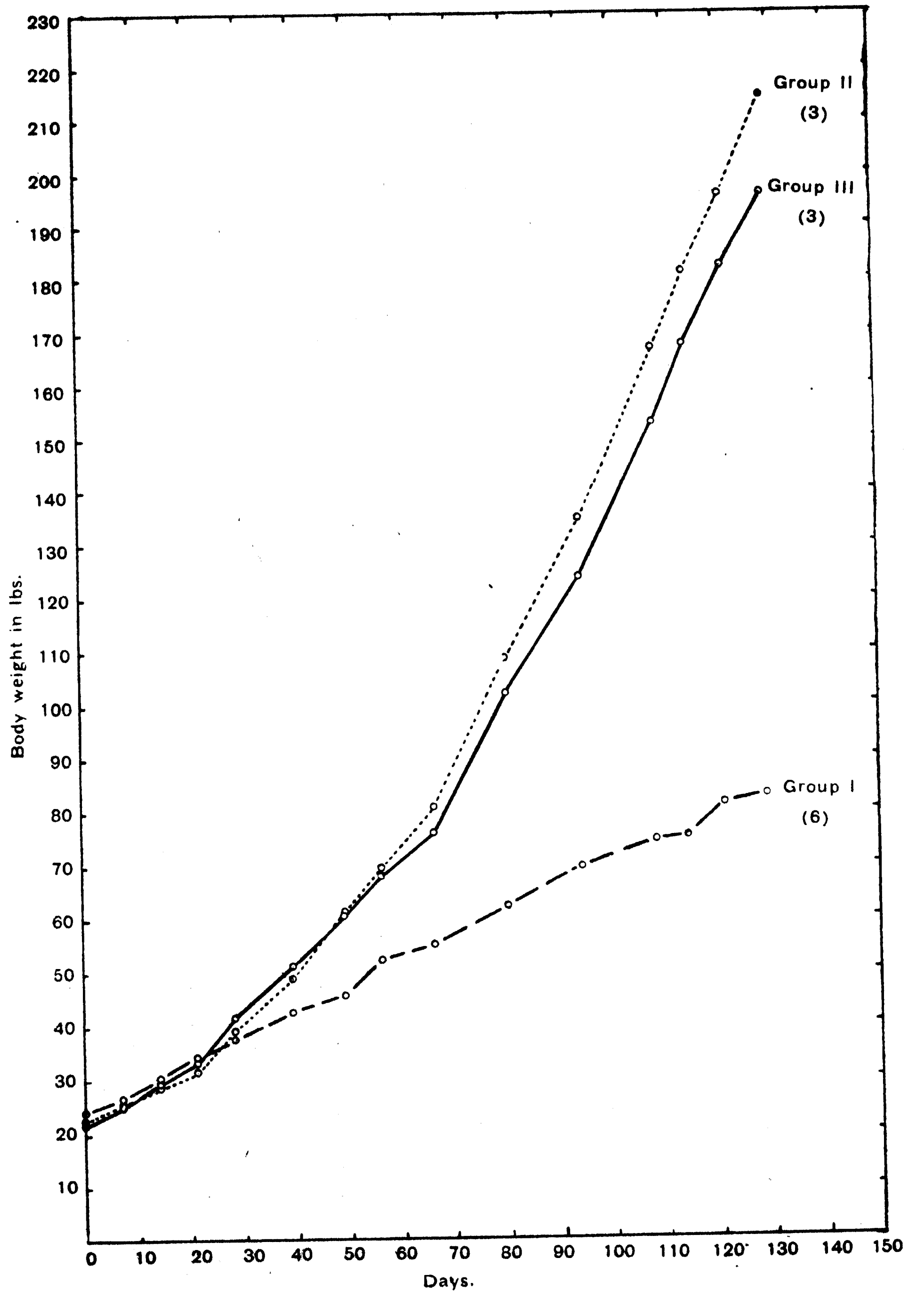

FIG. 1.—Graphs showing rate of growth of pigs in Groups I, II and III. Group I=low vitamin A (white maize); Group II=optimum (yellow maize meal); Group III=optimum (lucerne meal). These curves show that pigs on the vitamin A deficient ration made normal growth for the first month on experiment. For composition of rations see Table 1. The numbers in brackets show the number of animals considered.

Fig. 2.—Three male litter mates after 129 days on experiment. Pig in centre (No. 44, Gr. I) received the vitamin A deficient ration; pig on right (No. 33, Gr. II) received carotene in yellow maize, and one on left No. 35, Gr. III) received carotene in lucerne meal.

Fig. 3.—Three male litter mates after 129 days on experiment. Pig in centre (No. 30, Gr. I) received the vitamin A deficient ration; pig on right (No. 31, Gr. II) received carotene in yellow maize and one on left (No. 32, Gr. III) received carotene in lucerne meal.

Fig. 4.—Pig 42 ♀, Gr. I. Paralysis of hind-quarters after 129 days on the vitamin A deficient ration.

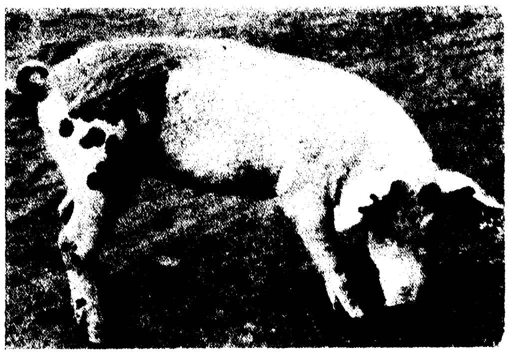

Fig. 5.—Pig ♀. Animal able to stand again after receiving 100 ml. cod liver oil daily for 10 days.

Fig. 6.—Pig 30 ♂, Gr. I. Pig shows scoliosis and paralysis of forequarters after 186 days on the vintamin A deficient ration.

Fig. 7.—Pig 30 ♂. Animal able to stand again after receiving cod liver oil for 18 days. (For particulars see Table 5.)

Fig. 8.—Pig 30 ♂. Animal still shows scoliosis after 102 days of treatment with cod liver oil. (See Table 5.)

Fig. 9.—Pig 53 ♀, Gr. I. Animal made poor growth and appeared thin and leggy after 129 days on the vitamin A deficient ration.

Fig. 10.—Pig 53 ♀, after receiving 25 ml. cod liver oil daily for 121 days.

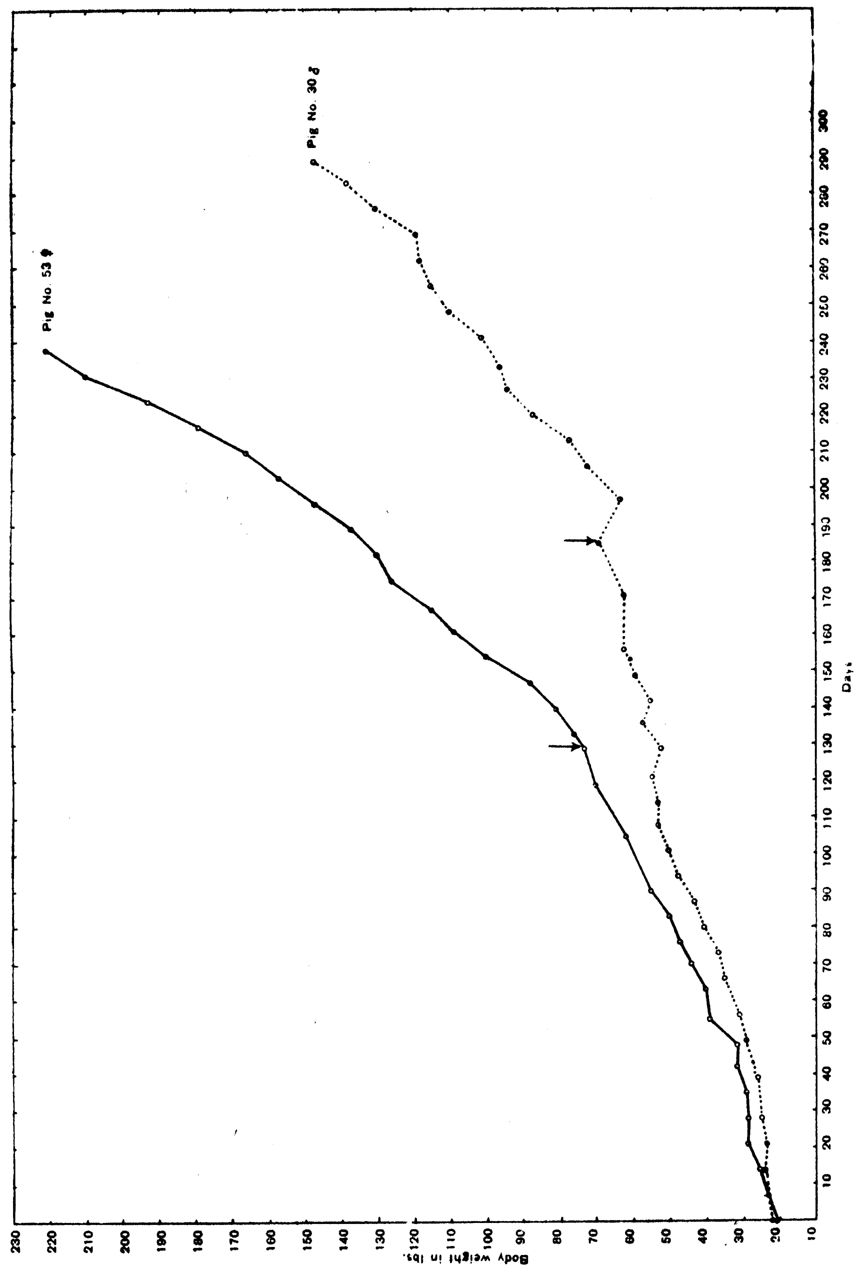

FIG. 11.—Graphs showing rate of growth of pigs 30♂ and 53♀ on vitamin A deficient ration (Ration I) before and during cod-liver oil supplementation. ↓ Commencement of cod-liver oil feeding. Additions described in Table 5.

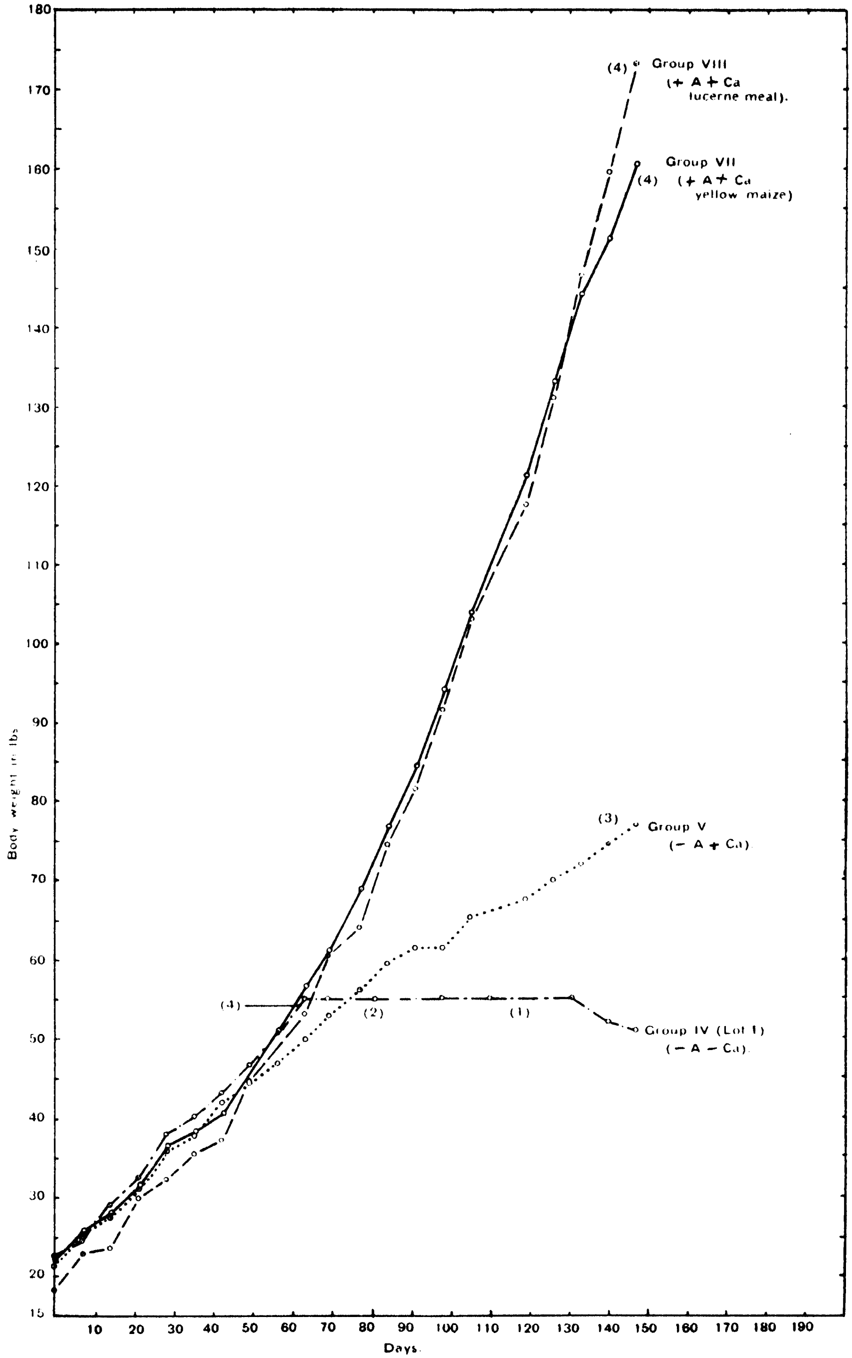

FIG. 12.—Graphs showing rate of growth of the pigs in Groups IV, lot 1, V, VII and VIII. Group IV, lot 1 = − A − Ca; Group V = − A + Ca; Group VII = + A + Ca (yellow maize meal); Group VIII = + A + Ca (lucerne meal). The signs (−) = deficient; (+) = optimum; and A = provitamin A. For composition of rations see Tables 8 and 9. N = number of animals considered. These curves show that the pigs on the vitamin A deficient rations made normal growth for the first two months on experiment. (Groups IV and V.) Unfortunately two of the pigs in Group IV, lot 1, fractured their vertebral columns rather early in the experiment and the two remaining pigs in this group got infected with *Ascaris lumbricoides* which fact, no doubt, complicated the effects of the vitamin A and Ca deficient ration.

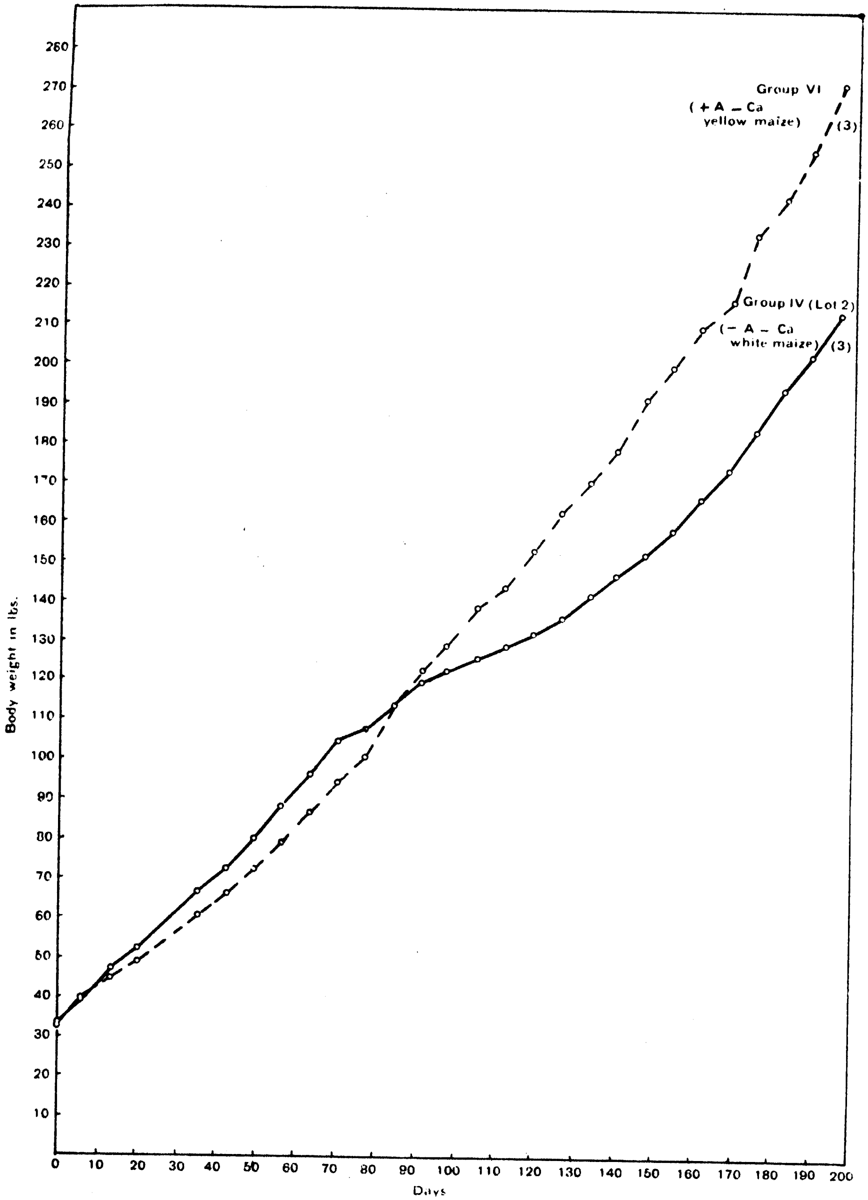

FIG. 13.—Graphs showing rate of growth of the pigs in Group IV, lot 2, and Group VI. Group IV, lot 2 = – A – Ca (white maize meal); Group VI = + A – Ca (yellow maize meal). The signs (–) = deficient; (+) = optimum; and A = provitamin A. For composition of rations see Tables 8 and 9. These curves show that the pigs on the vitamin A and Ca deficient ration made good growth for the first 80 days on experiment, whereas the pigs on the Ca deficient ration made good gains during the whole experimental period. N = number of animals considered.

Fig. 14.—Four hogs after 134 days on experiment. The two small pigs in centre (Nos. 39 and 43 Gr. V) were fed a vitamin A deficient ration of skim-milk, white maize and minerals; pig on left (No. 37, Gr. VII) received yellow maize, and one on right (No. 38, Gr. VIII) lucerne meal as the carotene containing ingredients.

Fig. 15.—Three gilts (litter mates) after 134 days on experiment. Pig on left (No. 50, Gr. IV, lot 1) was fed a Ca and vitamin A deficient ration of skim-milk and white maize. This animal also suffered from *Ascaris lumbricoides* infection. Pig on right (No. 55, Gr. V) was fed a vitamin A deficient ration of skim-mink, white maize and minerals, whereas the pig in centre (No. 59, Gr. VIII) received a normal ration of skim-milk, white maize, lucerne meal and minerals.

Fig. 16.—Gilts after 133 days on experiment. Pig on left (No. 81, Gr. IV, lot 2) received skim-milk and white maize, and pig on right (No. 82, Gr. VI) received skim-milk and yellow maize.

Fig. 17.—Hogs after 166 days on experiment. Pig on left (No. 83, Gr. IV, lot 2) received skim-milk and white maize, and one on right (No. 84, Gr. VI) received skim-milk and yellow maize.

Fig. 18.—Pig 85 ♂, Gr. IV, lot 2, with severe scoliosis after 255 days on a Ca and vitamin A deficient ration of skim-milk and white maize.

Fig. 19.—Pig 85, Gr. IV, lot 2, after receiving cod liver oil for 37 days. Animal has recovered almost completely from scoliosis. (For particulars see Table 13.)

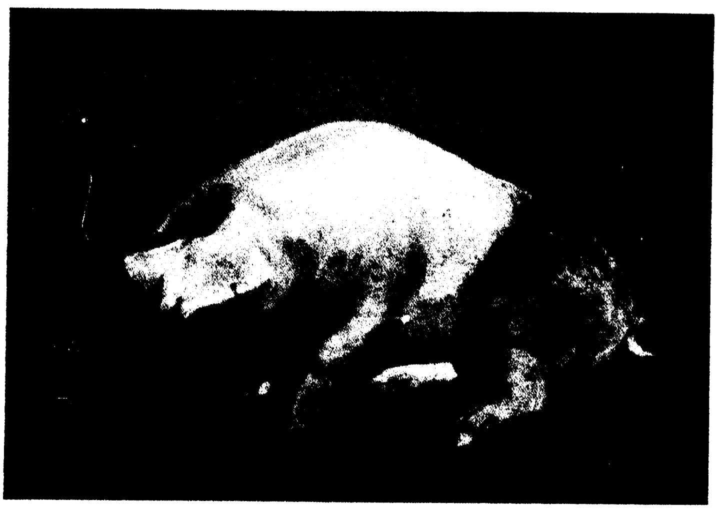

Fig. 20.—Pig 3, Gr. IV, lot 2, with partial "posterior paralysis" after 292 days on a Ca and vitamin A deficient ration of skim-milk and white maize.

Fig. 21.—Pig 83, Gr. IV, lot 2, after receiving cod liver oil for 37 days. (See Table 13.)

Fig. 22.—Pig 41 ♂, Gr. IV, lot 1. Paralysis of hindquarters as the result of a fractured vertebral column which happened 67 daws after commencement of experiment.

Fig. 23.—Pig 81 ♀, Gr. IV, lot 2. Nervous collapse after 279 days on a Ca and vitamin A deficient ration of skim-milk and white maize.

Fig. 24.—Pig 55 ♀, Gr. V. Posterior paralysis after 250 days on a vitamin A deficient ration of skim-milk, white maize and minerals.

Fig. 25.—Pig 43 ♂, Gr. V, after 316 days on a vitamin A deficient ration of skim-milk, white maize and minerals. Animal manifested a dirty, rough coat, runted appearance and drooping ears.

Fig. 26.—Natural cases. Animals paralysed, dragging hindquarters.

Fig. 27.—Natural cases. Typical posture when at rest.

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Domsiekte or Pregnancy Disease in Sheep—III.

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INTRODUCTION.

IN a previous communication, Groenewald *et al* (1941) reviewed the literature relating to domsiekte and reported the artificial production of the disease by suddenly reducing the ration of good conditioned ewes. The experiment to be recorded was undertaken to confirm the findings previously reported and to attempt treatment on the cases so produced.

EXPERIMENTAL PROCEDURE.

Forty 4 to 6 tooth merino ewes were fed a concentrated mixture of yellow maize meal and meat and bone meal, as described by us in a previous publication (Groenewald *et al* 1941). The total consumption of grain and green feed showed no marked deviation from that already recorded. All the sheep put on weight and averaged well over 100 lb. in weight at the time when the experiment started. At the beginning of the fifth month of pregnancy the concentrated ration was removed in all but the control group. The sheep were divided into five different groups in order to supply various supplements in the hope of finding a preventive dietetic factor. The experimental plan is given in Table 1.

TABLE 1.—*Treatment of Different Experimental Groups.*

Group 1.—(Control). To continue to receive a good ration.

Group 2.—To be given poor quality hay only from beginning of fifth month of pregnancy.

Group 3.—As for Group 2 except that ¼ lb. molasses supplemented daily.

Group 4.—As for Group 2 except that 2 oz. of a mixture of 80 per cent. bicarbonate of soda, 10 per cent. bone meal and 10 per cent. salt be supplemented daily.

Group 5.—Non-pregnant ewes treated as Group 2.

An equal number of sheep was not placed in each group as it was not considered necessary to have many controls and any sheep that were found not to be pregnant fell automatically into Group 5. Group 2 was made the largest as cases were required for treatment.

The results of the experiment will be found briefly tabulated in Tables 2 and 3.

Table 2.

Pregnant Sheep.

Group.	Sheep No.	Date Ration Cut.	Date of first Symptoms.	Degree of Symptoms.	Blood Chemistry. Acetone, Total.	Blood Chemistry. Glucose.	Date Lambed or Aborted.	Date Died.	Post Mortem Findings.	Remarks.
I Control.	27	—	14/7	Coma	High	Low	(L) 14/7	15/7	Typical domsiekte	Voluntary starvation from about 8/7 following intercurrent temp. of 104°.
	16	—	—	None	Normal	Normal	(L) 28/7	—	—	Normal.
	41	—	—	None	Normal	Normal	—	—	—	Non-pregnant.
	1	—	—	None	Normal	Normal	(L) 27/7	—	—	Normal.
	6	—	—	None	—	—	—	—	—	Non-pregnant.
II Hay only.	2	28/7	30/7	Typical spasms	—	—	(L) 17/8	—	—	Treated, recovered.
	14	17/6	26/6	Nervous, blind	High	Low	—	7/7	Too decomposed	Domsiekte.
	40	18/6	24/6	Unsteady, blind	High	Low	—	26/6	Typical domsiekte	Twin pregnancy; voluntarily off feed before ration cut.
	18	24/6	2/7	Nervous, blind	High	Low	(L) 17/7	—	—	Treated, recovered.
	30	26/6	4/7	Listless	High	Low	(A) 19/7	—	—	Recovered after abortion.
	33	27/6	3/7	Nervous, blind	—	—	—	3/7	Typical domsiekte	Twin pregnancy.
	39	1/7	7/7	Nervous, blind	—	—	—	8/7	Typical domsiekte	—
	4	17/6	24/6	Listless	High	Low	—	8/7	Toxaemia, dead foetus	—
	38	5/7	10/7	Fits, blind	—	—	—	29/7	Dead foetus, septic metritis	Treated 14/7.
	31	17/6	4/7	Nervous	High	Low	—	20/7	Typical domsiekte, dead foetus?	—
III Molasses.	26	17/6	—	None	Normal	Normal	—	1/7	Pneumonia	—
	3	27/6	1/7	Nervous	Normal	Normal	(L) 5/7	29/7	Domsiekte and metritis	Lamb died 24 hours old.
	11	27/6	—	None	—	—	—	12/7	Toxaemia, dead foetus	Found *in extremis*.
	36	1/7	—	None	—	—	(L) 20/7	—	—	Ewe had no milk.
	25	23/6	—	None	Normal	Normal	(L) 21/7	—	—	—
IV Alkali.	32	16/6	25/6	Dull, blind	High	Low	(A) 3/7	—	—	Aborted twins, recovered.
	23	5/7	17/7	Listless	—	—	—	18/7	Typical domsiekte	—
	5	18/6	27/6	Nervous, blind	High	Low	(A) 9/7	10/7	Oedema lungs, fatty liver	—
	24	3/7	—	None	—	—	(L) 20/7	—	—	—
	17	5/7	—	None	—	—	(L) 1/8	—	—	Dystokia, recovered.

N.B.—(L): Lambed. (A): Aborted.

TABLE 3.

Non-pregnant Ewes.

Sheep No.	Date Ration Cut.	Blood Chemistry.		Date Died.	Post-mortem Findings.	Remarks.
		Acetone.	Glucose.			
28	21/6	1/7 Normal 21/8 High.	Low...... Normal...	26/8	Domsiekte and enteritis	—
9	1/7	—	—	20/8	Typical domsiekte....	—
25	29/10	High......	Low.......	3/1	Typical domsiekte....	Starved again after lambing.
22	27/6	—	—	—	—	Discharged 28/7.
35	5/7	—	—	—	—	Discharged 28/7.
7	16/9	High......	Low.......	30/12	Typical domsiekte....	—
8	16/9	High......	Low.......	7/11	Too decomposed......	—
12	16/9	High......	Low.......	23/10	Too decomposed......	—
19	25/9	High......	Low.......	12/4	Typical domsiekte....	—
20	25/9	Normal....	Normal....	23/10	Typical domsiekte....	Died suddenly.
21	15/10	Normal....	Normal....	29/1	Typical domsiekte....	Died suddenly.
29	15/10	Normal....	Normal....	27/11	Typical domsiekte....	Died suddenly.
34	17/7	High......	Normal....	26/8	Typical domsiekte....	Died suddenly.
37	15/10	Normal....	High......	17/11	Pneumonia...........	—

NOTES.—None of the above sheep showed symptoms of domsiekte. For details of post-mortem findings designated "Typical Domsiekte" see paragraph on this subject.

RESULTS.

Controls.—Of five, two were non-pregnant. One (27) contracted an intercurrent disease when 169 days pregnant and went off her feed. She went into a coma 6 days later and gave birth to a weak lamb, dying soon afterwards. The post-morten showed typical domsiekte.

The other two lambed normally.

Group 2.—These sheep were given dry veld grass only at four months after service. Those that proved to be non-pregnant are dealt with under the non-pregnant group. Of 10 pregnant sheep suddenly reduced in ration as above, all 10 showed symptoms of domsiekte in periods ranging from 2-9 days (average 6 days). Two of these sheep had twin pregnancies, but this did not accelerate the disease as both went down in 6 days. Sheep No. 40 went off feed voluntarily before the ration was cut.

Group 3.—Five pregnant ewes were treated as above except that they were dosed with ¼ lb. molasses daily. Only one of these showed slight nervous symptoms on one day. Three lambed but two had very weak lambs and one of the ewes died subsequently of metritis. One died of toxaemia from a dead foetus *in utero* and the fifth died of intercurrent pneumonia.

One sheep of this group requires special mention, namely, sheep No. 25 (see appendix III for full details). This animal was treated as the others in the group and lambed. It was then fed the full ration for three and a half months, the lamb being removed. After this it was again put on to dry veld hay only and, as a non-pregnant animal, showed the typical acetonaemia and hypoglycaemia of domsiekte.

The dosing of molasses, therefore, appears to have had some effect in preventing the symptoms, but the dose given was not enough to cause normal lambing or to maintain weight. It would be impossible to give more as even this dose caused severe diarrhoea. In the three cases where blood analyses were done, the sugar appeared to prevent the formation of ketone bodies.

Group 4.—Five pregnant sheep were treated as above, but were also given a mixture consisting of 80 per cent. $NaHCO_3$, 10 per cent. bone meal and 10 per cent. salt. One ounce of this was dosed twice a day. Three of these five sheep showed symptoms. One lambed normally and the fifth died of toxaemia due to the presence of a retained disintegrating foetus. It is interesting to note that the alkali treatment had no antiketogenic effect and was useless in the prevention of domsiekte.

Group 5.—Fourteen non-pregnant ewes were also treated as above. Of these none showed clinical symptoms of domsiekte, but 12 died in periods ranging from 28 days to 199 days (average 69 days). Of the 12 sheep that died, two were too decomposed for diagnosis when brought for post-mortem, but eight showed typical domsiekte lesions. It will, therefore, be seen that non-pregnant ewes are much more resistant than pregnant ewes to a sudden reduction of ration but, provided that they are starved for long enough periods the same pathological changes take place. (For detailed records of individual sheep the reader is referred to appendices I to IV.)

Loss in Weight.

Adiposity has long been considered an important factor in the aetiology of domsiekte. The sheep in this experiment were fattened for a long period prior to the eyperiment, and were all in super prime condition.

It was thought that the rate of loss might determine the occurrence of domsiekte. The pregnant ewes lost weight at an average rate of approximately 1·0 lb. per day, but no significant difference could be found between the rate of loss of weight of those showing symptoms and of those which did not.

The average weight of the non-pregnant sheep used in the domsiekte experiment was 122 lb. and their average daily loss was 0·5 lb. These sheep on post-mortem all showed large amounts of fat still present in the depots, although they had been living on poor grass hay for long periods, in one case 199 days (sheep 19). It is, therefore, obvious that, in the absence of a sufficient balanced diet, sheep are incapable of utilizing stored fat.

A completely different picture was seen in another similar experiment where three non-pregnant ewes in moderate condition were also put on to the same grass hay only. At the commencement of this experiment the ewes averaged 86 lb. in weight. As the ewes used in these two experiments were of exactly the same type and were in fact all picked from the available ewes on the station it can be fairly assumed that the difference in initial weight between the two groups was almost entirely due to fat. When the lower conditioned ewes were put on to the grass diet they only lost an average of three pounds per sheep in six months. One was actually alive ten months after going on to the grass hay only and was discharged, having lost 11 lb. in weight. The other two died of cachexia and on post-mortem showed no fat in the body, there being only a gelatinous atrophy of the fatty tissues.

It is, therefore, obvious that the amount of adipose tissue in the body has a very marked effect on the reaction to a sudden drop in nutritional intake.

Clinical Symptoms.

The symptoms seen in the pregnant ewes were typical of domsiekte, namely, dullness, twitching of the ears and spasms of the body, blindness, usually of one eye, and coma as described in our previous publication. (Groenewald *et al* 1941.) A point of interest is the frequency of apparent death of the foetus with partial recovery of the ewe but without abortion. It can be said that when a ewe shows symptoms of domsiekte and then lingers on without either lambing or aborting, it can be assumed that the foetus is dead and the ewe will eventually die of toxaemia. Reference to the prevalence of abortion among domsiekte sheep was made in the last article. Further reference to the death of the foetus will be made under the discussion of the chemical findings in the blood.

The Blood Chemistry.

(For details of analyses see appendices I to IV.) As will be seen from table 2, all the pregnant sheep showed the usual blood chemical changes when the symptoms developed, i.e. a rise in ketone bodies and a fall in blood sugar with the N.P.N. not affected.

A peculiar phenomenon appeared in pregnant sheep 4, 14 and 31 and in several of the non-pregnant animals, namely that after a period of hypoglycaemia and ketonaemia the blood-sugar shot up well above normal figures with a simultaneous drop in the ketone bodies and rise in the N.P.N.

In the case of the pregnant sheep this can be explained by assuming that the foetus died at this point but was not expelled. Evidence of this was found at post-mortem. The sudden drop in metabolic requirements subsequent to the death of the foetus would explain the rise in blood sugar and consequent drop in ketone bodies, while the rise in N.P.N. would be due to absorption of break-down products from the foetus aggravated by the gradually mounting disfunction of the kidneys following on degenerative changes. It must be remembered, however, that non-pregnant sheep also showed the same phenomenon. An alternative explanation is that at a certain point there is a sudden large scale catabolism of protein for the production of the much needed carbohydrates. It is probable that both factors come into play.

Pathological Findings.

The macroscopical pathological findings were as described in Number II of this series, being briefly: gross fatty changes in the liver and adrenal cortex, obesity with marked fat necrosis especially of the perirenal fat and atrophy of the lymphatic tissue. The alimentary tract usually shows atrophy of the ruminal wall, small intestines empty and the presence of hard mucous covered faeces in the large intestine.

Histopathology.

The histological changes noted were as previously reported (Groenewald *et al*, 1941). The only additional lesion noted was in the lymph nodes.

As previously reported the drop in circulating lymphocytes was associated with an atrophy of the lymph-nodes, affecting especially the germ centres, and a shrinking of the malpighian bodies of the spleen. From all cases of the present series which died, specimens were taken of the prescapular, precrural, mediastinal and mesenteric lymph glands. All these were very much smaller than normal but the most marked decrease was noted in the external glands, i.e. prescapular and precrural.

On histological examination of these nodes it was found that the germ centres were markedly decreased in size and density. Islands of typical foam cells were noted in the cortex, occupying the positions of the disappearing germ centres and obviously consisting of swollen and hypertrophied cells derived from the reticulum of the centres. The cytoplasm of these foam cells showed typical vacuolation with the nuclei often pressed to one side. Often no division could be noted between several adjacent cells, giving the appearance of multinuclear giant cells. The vacuoles of the cytoplasm did not stain with routine sudan III methods but when the new acetic-carbol-sudan III technique of Jackson was applied, the vacuoles took on a bright red colour. There was also a marked hypertrophy and desquamation of the littoral cells of the sinuses resulting in these channels being packed with macrophages also exhibiting foamy cytoplasm. With routine Sudan III methods many of these vacuoles were refractory but with Jackson's technique the cytoplasm was seen to be packed with red-stained globules of varying sizes.

The exterior lymph nodes showed the alteration of the follicles to a greater degree than did the interior ones, but the latter showed isolated foam centres in the cortex and marked desquamation of the littoral cells of the sinuses, together with a varying degree of hyperaemia.

The changes noted are, therefore, an atrophy of lymphoid tisssue together with a typical reticulo-endothelial reaction of lymph nodes associated with phagocytosis of fats. Curiously enough the latter reaction was not noted in the spleen. In view of the known abnormality of fat catabolism in domsiekte, this lesion may be of great significance and its study may help to elucidate the question of normal fat catabolism in sheep. Identical lesions have been noted in the lymph nodes of sheep dead of domsiekte in naturally occurring outbreaks from the field.

The Blood.

The drop in circulating lymphocytes and rise in neutrophiles which was described in our last article was again noted. The average differential count for sheep showing symptoms was:—neutrophiles 56%, lymphocytes 39% with no significant change in the leucocyte total.

Correlation between Acetonaemia on the One Hand and Clinical Symptoms on the Other.

Among the pregnant ewes in all cases where symptoms were noted the blood showed a fall in glucose and a rise in ketone bodies. Of the eleven non-pregnant ewes on which chemical data is available, however, six showed identical and equally severe changes without showing clinical symptoms.

The symptoms of domsiekte cannot therefore be attributed to the acetonaemia or hypoglycaemia alone. From this experiment it would appear that pregnancy is necessary for the development of the clinical picture of domsiekte but, as previously reported (Groenewald, *et al* 1941), typical symptoms have been seen in non-pregnant ewes.

The Correlation of Chemical Findings to Pathological Changes.

All the pregnant sheep which showed hypoglycaemia and acetonaemia were found on post-mortem to have extensive fatty changes in the liver and other typical changes associated with domsiekte.

Turning again to the non-pregnant ewes, however, we find that these same changes appeared in sheep that had never shown typical chemical changes. The anatomical pathological findings recorded to date, therefore, cannot be directly correlated with the chemical pathology.

Correlation between Symptoms and Pathological Findings.

Once again we find among the non-pregnant ewes that typical pathological changes did not always produce clinical symptoms.

Treatment.

Three pregnant sheep were treated with intravenous injections of 10 gm. glucose, 3333 i.u. Thiamin hydrochloride (Abbott) and 1 mgm. lentin subcut. All three showed improvement but one died subsequently of toxaemia from a dead foetus. Particular reference may be made to the history of sheep 18. (See appendix II.) This animal showed typical symptoms of domsiekte 10 days after the cut in ration and was treated on the twelfth day when in coma. This sheep ate grain offered the following day and in three days the blood chemistry was normal. The ewe lambed normally.

Similar treatment has been used by the authors in a naturally occurring outbreak with some success. It is considered that the main object to be attained in any treatment is to get purgation. It has been noted that ruminal atony and constipation of the large intestine is an almost constant finding in cases of domsiekte and no permanent improvement can be expected till this is relieved. Lentin acts well but 120 c.c. of raw linseed oil has also been found effective. Rapidly absorbable carbohydrate such as glucose or sugar is also obviously indicated.

Summary.

(1) It has been confirmed that a sudden and drastic reduction in diet of fat ewes in the fourth month of pregnancy causes typical domsiekte or pregnancy disease. The condition was evinced clinically, chemically and pathologically.

(2) Similar treatment of fat, non-pregnant ewes caused the same changes in blood chemistry as seen in pregnancy disease in some, and post-mortem findings typical of the disease in others. No clinical symptoms were observed in non-pregnant ewes.

(3) In the non-pregnant group no correlation could be found between chemical changes, post-mortem findings and clinical symptoms.

(4) The non-pregnant ewes survived for a very much longer period than the pregnant ewes.

(5) A hitherto undescribed pathological finding, namely hypertrophy of the retico-endothelial system of the lymph nodes is recorded.

(6) Indications are given that the use of alkaline dosing has no effect on the formation of acetonaemia.

(7) Treatment by means of rapidly active purgatives and sugars is indicated.

LITERATURE.

As a full review and list of literature relating to domsiekte has been given in a previous publication (Groenewald *et al* 1941), it is not considered necessary to repeat it here. The reader is, therefore, referred to the following:—

CLARK, R., AND GROENEWALD, J. W. (1941). Pregnancy Disease or Domsiekte in Ewes. *Jour. South Afric. Med. Vet. Assoc.*, Vol. 12, No. 4, pp. 97-102.

GROENEWALD, J. W., GRAF, H., AND CLARK, R. (1941). Domsiekte or Pregnancy Disease in Sheep 1.—A review of the Literature. *Onderstepoort Jl.*, Vol 17 (1 and 2), pp. 225-244.

GROENEWALD, J. W., GRAF, H., BEKKER, P. M., MALAN, J. R., AND CLARK, R. (1941). Domsiekte or Pregnancy Disease in Sheep II. *Onderstepoort Jl.*, Vol. 17 (1 and 2), pp 245-296.

APPENDIX I.

Details of Control Group.

No.	Date.	Body Weight. (lb.).	Blood Analyses. Biochemical, mgm. Per cent. N.P.N.	Sugar.	Total Acetone.	Counts. Total Leuc. per c.mm.	Per cent. L.	Remarks.
27	18/6	—	34·1	43·9	3·9	—	—	—
	24/6	121	—	—	—	—	—	—
	3/7	—	30·0	41·3	18·2	—	—	—
	8/7	—	27·3	22·2	31·7	—	47	Ill, voluntary starvation, temperature 104°.
	11/7	—	27·2	21·3	42·6	—	—	High acetone, low sugar.
	14/7	—	—	—	—	—	—	Lambed.
	15/7	—	—	—	—	—	—	Died, typical domsiekte P.M.
16	24/6	117	—	—	—	—	—	—
	3/7	—	30·2	43·9	3·9	—	—	—
	9/7	—	35·6	50·0	3·9	—	49	—
	15/7	—	33·6	48·1	3·5	6,300	48	—
	18/7	—	—	—	—	5,600	41	Normal acetone and sugar.
	24/7	—	35·1	37·6	6·6	—	—	—
	28/7	—	—	—	—	—	—	Lambed.
	30/7	—	31·6	52·6	4·6	—	—	—
	20/8	—	30·0	52·9	2·9	—	—	—
1	24/6	149	—	—	—	4,600	63	—
	2/7	—	33·3	55·6	3·9	4,500	64	—
	8/7	—	33·6	60·2	2·9	—	68	Normal acetone and sugar.
	14/7	—	30·0	50·0	2·7	5,200	59	—
	18/7	—	—	—	—	5,200	50	—
	22/7	—	—	—	—	—	—	Lambed.
	24/7	—	33·3	46·5	3·5	—	—	—
	30/7	144	35·3	64·5	4·5	—	—	—
41	24/6	118	—	—	—	7,000	62	—
	4/7	—	45·4	51·0	4·1	7,800	51	—
	10/7	—	49·6	49·5	2·7	4,900	61	—
	15/7	—	40·0	39·2	2·3	6,500	54	—
	17/7	—	36·1	45·5	2·5	—	—	Normal acetone and sugar.
	24/7	—	40·8	49·5	3·9	—	—	—
	30/7	—	42·9	54·1	4·3	—	—	Non-pregnant.
6	24/6	130	—	—	—	4,300	72	—
	2/7	—	—	—	—	6,900	73	—
	10/7	—	—	—	—	—	63	—
	15/7	—	—	—	—	5,700	71	—
	28/7	140	—	—	—	—	—	Non-pregnant.

APPENDIX II.

Detailed History of Individual Pregnant Sheep.

No.	Date.	Body Weight. (lb.).	Biochemical Blood. N.P.N.	Biochemical Blood. Sugar.	Biochemical Blood. Total Acetone.	Symptoms.	Blood. Total Leuc. per c.mm.	Blood. Per cent. L.	Remarks.
2 Gr. 2	28/7	132	—	—	—	—	—	—	Put off ration.
	30/7	128	—	—	—	Spasms	—	—	Treated Lentin, glucose and Vitamin B.
	1/8	—	—	—	—	Recovered	—	—	Refuses food, force fed.
4 Gr. 2	16/6	—	31·3	47·2	3·3	—	—	—	—
	17/6	163	—	—	—	—	—	—	Ration cut.
	24/6	151	26·1	29·4	17·0	Dull, blind	7,800	19	—
	25/6	—	24·8	28·5	24·8	Improved	—	—	Sugar low, acetone high.
	26/6	—	—	—	—	—	4,000	40	—
	27/6	—	28·6	34·5	42·4	As before	—	—	—
	30/6	—	41·6	51·0	38·7	—	7,600	26	Foetus dead ?
	1/7	142	—	—	—	As before	—	—	—
	2/7	—	102·7	100·0	24·0	As before	—	—	N.P.N. and sugar high.
	3/7	—	—	—	—	—	8,500	14	—
	4/7	136	146·4	100·0	5·8	As before	—	—	—
	7/7	131	193·6	72·5	9·3	—	—	19	N.P.N. and sugar high; acetone low.
	9/7	—	—	—	—	—	—	—	Died. Toxaemia decomposing foetus.
14 Gr. 2	16/6	—	30·0	46·7	4·6	—	—	—	—
	17/6	115	—	—	—	—	—	—	Ration cut.
	24/6	110	27·1	29·2	22·0	—	—	—	—
	25/6	—	25·0	19·2	34·6	—	—	—	Sugar low, acetone high.
	26/6	106	—	—	—	Listless, blind	6,000	41	—
	27/6	—	25·6	31·8	37·3	Listless, blind	6,700	43	—
	30/6	—	30·0	23·2	54·2	—	—	—	—
	1/7	98	33·3	31·7	68·0	As above	—	—	N.P.N. and sugar rising with high acetone Foetus dead ?
	4/7	98	39·8	49·5	70·0	—	16,200	10	—
	7/7	—	—	—	—	—	—	—	Died. Too decomposed for post-mortem.
18 Gr. 2	24/6	142	30·9	50·0	4·8	—	—	—	Ration cut.
	1/7	132	30·5	21·7	24·8	—	—	—	Sugar low, acetone high.
	2/7	132	—	—	—	Nervous	5,400	55	—
	3/7	—	—	—	—	Blind, dull	—	—	—
	4/7	—	27·9	25·7	39·5	Coma	—	—	Treated glucose and B_1.
	5/7	—	—	—	—	Eating	—	—	—
	8/7	—	25·8	55·0	6·2	—	—	64	Sugar and acetone normal.
	11/7	—	—	—	—	—	7,600	61	—
	17/7	—	—	—	—	—	—	—	Lambed normally.

APPENDIX II—(*continued*).

No.	Date.	Body Weight. (lb.).	Biochemical Blood. N.P.N.	Biochemical Blood. Sugar.	Biochemical Blood. Total Acetone.	Symptoms.	Blood. Total Leuc. per c.mm.	Blood. Per cent. L.	Remarks.
30 Gr. 2	26/6	127	32·2	44·6	3·7	—	7,100	53	Put off ration.
	30/6	—	—	—	—	—	4,400	58	—
	1/7	121	26·1	25·9	18·6	—	—	—	Sugar low, acetone high.
	4/7	118	—	—	—	Dull and listless..........	6,500	54	—
	5/7	115	—	—	—	—	—	—	—
	7/7	—	—	—	—	—	5,100	71	—
	10/7	—	—	—	—	—	5,700	55	—
	16/7	—	—	—	—	—	7,800	63	
	19/7	—	—	—	—	—	—	—	Aborted and recovered.
31 Gr. 2	25/6	—	25·4	46·7	3·7	—	—	—	—
	27/6	126	—	—	—	—	—	—	Put off ration.
	28/6	—	—	—	—	—	4,100	50	—
	1/7	120	27·6	25·3	12·4	—	—	—	—
	2/7	—	—	—	—	—	6,700	44	—
	4/7	116	—	—	—	Slight nervous............	—	—	—
	7/7	—	—	—	—	Dull and listless..........	2,800	43	—
	8/7	—	31·6	27·2	41·8	—	—	—	Sugar low, acetone high.
	9/7	—	—	—	—	Nervous..................	—	—	—
	10/7	—	40·8	43·5	61·5	—	5,500	19	Acetone high, sugar and N.P.N. rising, foetus dead ?
	12/7	—	57·2	74·6	77·0	—	—	—	—
	15/7	105	100·5	111·1	38·7	—	—	—	—
	16/7	—	—	—	—	—	12,900	10	—
	17/7	—	87·0	87·7	7·4	—	—	—	N.P.N. and sugar high, acetone low.
	20/7	—	—	—	—	—	—	—	Died. Typical domsiekte P.M. Foetus dead and disintegrating.
33 Gr. 2	27/6	139	—	—	—	—	7,100	43	Put off ration.
	1/7	134	—	—	—	—	—	—	—
	3/7	130	—	—	—	Marked nervous..........	6,200	38	Died. Typical domsiekte P.M. Twin pregnancy.
38 Gr. 2	5/7	117	—	—	—	—	—	—	Put off ration.
	8/7	115	—	—	—	—	—	36	—
	10/7	—	—	—	—	Sudden fit..............	—	—	—
	13/7	—	—	—	—	Nervous, blind right eye..	—	—	—
	14/7	—	—	—	—	Nervous.................	—	38	Glucose, B_1 and Lentin.
	15/7	—	—	—	—	Calm, drinks, does not feed	—	—	
	16/7	—	—	—	—	Passing soft faeces.......	—	—	Dosed, ½ lb. molasses.
	17/7	—	—	—	—	Stiff.....................	5,700	27	Dosed meatmeal.
	29/7	—	—	—	—	—	—	—	Died. Dead foetus and Toxaemia.
39 Gr. 2	1/7	139	—	—	—	—	8,000	47	Put off ration.
	3/7	—	—	—	—	—	5,100	37	—
	7/7	129	—	—	—	Loss of balance and blind.	7,000	25	Died. Typical domsiekte P.M.

APPENDIX II—(*continued*).

No.	Date.	Body Weight. (lb.).	Biochemical Blood. N.P.N.	Biochemical Blood. Sugar.	Biochemical Blood. Total Acetone.	Symptoms.	Blood. Total Leuc. per c.mm.	Blood. Per cent. L.	Remarks.
40 Gr. 2	18/6	112	33·0	28·4	42·9	—	—	—	Voluntarily off feed before ration cut.
	20/6	—	25·2	23·5	46·4	—	—	—	—
	24/6	—	—	—	—	Unbalanced	5,000	63	—
	25/6	—	29·4	18·4	42·9	Blind, unsteady	8,800	42	—
	26/6	—	—	—	—	—	—	—	Died. Typical domsiekte P.M. Twin pregnancy.
3 Gr. 3	27/7	131	22·7	46·3	4·3	—	—	—	Put off ration, plus ¼ lb. molasses.
	1/7	128	21·6	40·3	7·4	Nervous	—	—	—
	2/7	—	—	—	—	Improved	—	—	—
	3/7	—	—	—	—	—	5,800	24	—
	7/7	—	—	—	—	—	4,400	25	—
	8/7	122	29·7	44·6	6·8	—	—	—	Acetone, sugar and N.P.N. Normal.
	11/7	—	27·5	46·9	4·6	—	5,900	37	—
	12/7	—	—	—	—	—	—	—	Molasses stopped.
	14/7	—	22·5	41·0	6·2	—	—	—	—
	15/7	112	—	—	—	—	5,100	26	Lambed, weak lamb.
	16/7	—	—	—	—	—	—	—	Lamb died.
	17/7	—	26·8	76·9	3·9	—	—	—	—
	21/7	—	—	—	—	—	—	—	Metritis.
	29/7	—	—	—	—	—	—	—	Died. Fatty liver and metritis.
11 Gr. 3	27/6	115	—	—	—	—	—	—	Put off ration, plus ¼ lb. molasses.
	2/7	111	—	—	—	—	4,200	41	—
	12/7	—	—	—	—	—	—	—	Died. Dead foetus. Toxaemia, no domsiekte.
26 Gr. 3	17/6	97	32·3	38·5	6·6	—	—	—	Ration cut plus ½ lb. molasses.
	24/6	95	20·5	30·9	5·6	—	—	—	—
	1/7	—	—	—	—	—	—	—	Died suddenly. Pregnant, pleuro-pneumonia, no domsiekte.
36 Gr. 3	1/7	122	—	—	—	—	—	—	Put off ration, plus ¼ lb. molasses.
	4/7	—	—	—	—	—	5,400	21	—
	12/7	108	—	—	—	—	—	—	Molasses stopped.
	14/7	—	—	—	—	—	8,200	21	—
	20/7	—	—	—	—	—	—	—	Normal lamb, no milk.
24 Gr. 4	3/7	111	—	—	—	—	6,100	75	Put off ration and alkali dosed.
	7/7	110	—	—	—	—	3,900	76	—
	11/7	—	—	—	—	—	7,000	45	
	12/7	—	—	—	—	—	—	—	Alkali stopped.
	16/7	101	—	—	—	—	5,400	44	—
	20/7	—	—	—	—	—	—	—	Lambed normally.
	29/7	92	—	—	—	—	—	—	Put on full ration.
	7/8	—	—	—	—	—	—	—	Died. Decomposed at P.M. but no domsiekte.

APPENDIX II—(*continued*).

No.	Date.	Body Weight. (lb.).	Biochemical Blood.			Symptoms.	Blood.		Remarks.
			N.P.N.	Sugar.	Total Acetone.		Total Leuc. per c.mm.	Per cent. L.	
32 Gr. 4	17/6	140	31·9	45·5	3·5	—	—	—	Ration cut and alkali dosed.
	24/6	130	30·3	29·9	19·9	—	—	—	—
	25/6	—	—	—	—	Dull, blind left eye.......	6,300	64	—
	26/6	—	25·0	22·0	36·4	Dull, blind left eye.......	—	—	Sugar low, acetone high.
	30/6	—	41·6	28·4	50·3	—	5,900	49	—
	1/7	121	—	—	—	Still as above............	—	—	—
	2/7	—	26·6	27·5	53·8	—	—	—	—
	3/7	—	—	—	—	—	—	—	Aborted, twins, kept on ration.
	4/7	—	24·0	55·5	46·8	Improved................	—	—	Sugar high.
	9/7	—	20·2	49·8	14·7	—	—	—	Acetone dropping.
	12/7	—	23·8	49·8	11·5	—	—	—	Alkali stopped.
	15/7	95	22·2	43·1	6·6	—	—	—	—
	16/7	—	—	—	—	—	9.700	58	—
	28/7	104	—	—	—	—	—	—	Discharged.
5 Gr. 4	18/6	108	25·6	42·0	4·3	—	—	—	Ration cut and alkali dosed.
	24/6	107	23·6	18·2	21·9	—	—	—	—
	27/6	102	19·5	25·4	28·6	Nervous, blind left eye....	—	—	—
	30/6	—	24·6	19·8	39·8	—	—	—	Sugar low, acetone high.
	1/7	100	23·1	18·6	44·9	—	—	—	—
	4/7	—	25·8	24·3	39·5	—	7.400	25	—
	7/7	—	25·2	22·4	44·5	—	—	—	Sugar low, acetone high.
	8/7	97	—	—	—	—	—	—	Aborted.
	9/7	—	30·0	46·9	19·3	—	—	18	Sugar normal, acetone dropping.
	12/7	—	—	—	—	—	—	—	Alkali stopped.
	16/7	—	—	—	—	—	—	—	Died suddenly, oedema lungs, not domsiekte.
17 Gr. 4	5/7	119	—	—	—	—	—	—	Put off ration and alkali dosed.
	8/7	121	—	—	—	—	—	68	—
	12/7	—	—	—	—	—	—	—	Alkali stopped.
	15/7	106	—	—	—	—	—	—	—
	18/7	—	—	—	—	—	8,000	39	—
	29/7	98	—	—	—	—	—	—	Offered full ration.
	1/8	—	—	—	—	—	—	—	Died dystokia, lamb dead and decomposed.
23 Gr. 4	5/7	137	—	—	—	—	—	—	Put off ration and alkali dosed.
	9/7	138	—	—	—	—	—	56	—
	12/7	—	—	—	—	—	—	—	Alkali stopped.
	17/7	—	—	—	—	Listless..................	—	—	—
	18/7	—	—	—	—	—	—	—	Died suddenly. Typical domsiekte P.M.

Appendix III.

Details Sheep No. 25.

Date.	Body Weight. (lb.).	Blood Analyses, mgm. (Per Cent.).			Remarks.
		N.P.N.	Sugar.	Total Acetone.	
20/6	—	26·1	46·9	5·0	A pregnant ewe in group 3.
23/6	99	—	—	—	Put off ration and ¼ lb. molasses dosed daily.
24/6	100	—	—	—	—
26/6	—	20·1	50·3	2·9	—
2/7	92	20·3	47·6	3·9	—
8/7	91	21·1	41·0	3·1	—
15/7	87	23·2	34·3	6·8	—
21/7	—	23·8	45.5	6·8	Lambed, showing normal blood picture.
28/7	81	—	—	—	Molasses stopped and given full ration, for 3½ months.
30/7	—	28·6	51·3	2·9	—
20/8	—	25·4	49·5	2·5	—
29/9	—	27·3	52·6	1·2	—
29/10	—	32·9	48·5	1·7	Ration changed to veldhay only.
17/11	—	33·6	39·4	10·4	—
3/12	—	33·3	47·6	11·6	—
12/12	—	29·7	35·7	38·3	—
17/12	—	33·3	19·6	54·9	High acetone and low sugar after 7 weeks.
19/12	—	35·3	22·2	56·8	—
22/12	—	40·0	47·6	15·5	N.P.N. and sugar rising, acetone dropping.
29/12	—	53·6	61·7	4·7	—
2/1	—	60·0	61·7	5·4	N.P.N. and sugar high, acetone normal.
3/1	—	—	—	—	Died, typical domsiekte P.M.

APPENDIX IV.

Details of Non-pregnant Ewes.

Sheep No.	Date.	Body Weight. (lb.).	Blood Analyses, mgm. (Per Cent.).			Remarks.
			N.P.N.	Sugar.	Total Acetone.	
7	15/9	—	38·0	49·5	2·5	Ration cut.
	25/9	96	33·3	37·3	3·7	—
	7/10	—	24·6	41·3	2·9	—
	27/10	87	27·3	43·5	3·3	—
	17/11	—	33·3	39·7	6·9	—
	9/12	—	40·0	28·4	24·4	Sugar low, acetone high.
	17/12	—	20·3	32·3	23·3	—
	24/12	—	23·1	34·4	25·5	—
	29/12	58	26·3	34·0	21·6	Died suddenly. Typical domsiekte lesions.
8	16/9	—	34·5	44·5	4·1	Ration cut.
	25/9	123	31·9	37·0	5·9	—
	7/10	—	24·0	34·5	6·6	—
	20/10	—	25·6	25·6	33·2	Sugar low, acetone high.
	31/10	111	25·0	41·3	22·4	—
	3/11	—	61·8	50·0	2·6	N.P.N. and sugar high, acetone low.
	7/11	—	—	—	—	Died suddenly. No P.M.
12	16/9	—	35·3	51·3	1·6	Ration cut.
	29/9	97	33·3	39·4	8·1	—
	13/10	—	24·0	31·3	13·2	Sugar low, acetone above normal.
	20/10	—	73·2	52·6	7·0	N.P.N. and sugar high.
	23/10	—	—	—	—	Died suddenly. No P.M.
19	25/9	148	38·9	48·0	2·0	Put off ration.
	16/10	—	24·8	45·5	4·1	—
	31/10	131	27·8	41·7	4·5	—
	12/11	—	17·5	41·7	3·5	—
	3/12	—	19·1	47·2	5·1	—
	23/12	—	21·0	43·5	2·7	—
	13/1	—	20·7	48·8	3·0	—
	3/2	—	14·7	42·6	5·3	—
	24/2	97	24·0	43·3	5·1	—
	9/3	—	13·6	48·8	4·7	—
	31/3	—	20·0	27·5	20·4	High acetone, low sugar, after 187 days.
	7/4	—	21·4	37·9	27·5	—
	10/4	—	30·0	62·5	7·4	High sugar, normal acetone.
	12/4	—	—	—	—	Died after 199 days. Typical domsiekte. P.M.
20	25/9	139	30·9	42·9	2·3	Put off ration.
	9/10	—	23·1	38·5	4·1	—
	23/10	—	35·3	30·7	5·0	Died suddenly, typical domsiekte lesions.
21	15/10	142	28·0	46·7	1·9	Put off ration.
	31/10	134	30·0	41·7	4·3	—
	6/11	—	20·0	45·5	4·3	—
	24/11	124	18·6	40·7	6·2	—
	3/12	—	20·7	50·3	5·4	—
	23/12	106	29·2	46·5	5·1	—
	13/1	—	20·0	48·1	4·7	—
	27/1	88	23·3	53·7	4·7	—
	29/1	—	—	—	—	Died. Typical domsiekte lesions.

APPENDIX IV—(*continued*).

Sheep No.	Date.	Body Weight. (lb.).	Blood Analyses, mgm. (Per Cent.).			Remarks.
			N.P.N.	Sugar.	Total Acetone.	
28	18/6	—	30·9	55·0	3·5	—
	21/6	134	—	—	—	Put off ration.
	24/6	132	33·3	37·6	3·9	—
	1/7	128	25·2	20·8	3·5	Low sugar, normal acetone.
	15/7	120	—	—	—	—
	28/7	113	—	—	—	—
	21/8	—	25·4	44·3	43·7	Normal sugar, high acetone.
	26/8	—	—	—	—	Died, domsiekte lesions and enteritis at P.M
29	15/10	131	30·0	47·6	2·5	Put off ration.
	31/10	125	37·0	43·1	8·1	—
	6/11	—	21·2	40·5	2·3	—
	26/11	—	60·0	40·3	3·0	—
	27/11	—	—	—	—	Died. Typical domsiekte lesions.
34	24/6	108	—	—	—	—
	17/7	—	31·4	44·3	3·5	Put off ration.
	21/7	—	45·4	32·7	4·5	—
	28/7	103	36·0	33·1	5·0	—
	21/8	—	60·0	59·5	31·5	High acetone with N.P.N. and sugar rising.
	26/8	—	—	—	—	Died, typical domsiekte P.M.
37	15/10	149	46·2	49·0	1·9	Put off ration.
	30/10	131	31·6	45·4	4·3	—
	12/11	—	120·0	69·0	2·9	—
	15/11	—	324·0	130·0	2·3	—
	17/11	—	—	—	—	Died pneumonia.

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Domsiekte or Pregnancy Disease in Sheep IV.—The Effect of Obesity on the Reaction of Sheep to a Sudden Reduction in Diet.

By R. CLARK, Section of Pathology, Onderstepoort.

INTRODUCTION.

IN continuation of the work on "domsiekte" or Pregnancy Disease in Sheep (Groenewald *et al*, II, 1941) a further experiment on the effect of a sudden cut in the ration has been completed. It has been reported that if the diet of fat pregnant sheep is suddenly reduced, typical pregnancy disease is rapidly produced. Identical changes also took place in non-pregnant ewes under the same conditions but the period of semi-starvation required was very much longer. It has also been shown that in typical Pregnancy Disease there is a marked reduction in the number of circulating lymphocytes. This phenomenon, together with the production of "Pregnancy Disease" in non-pregnant ewes, appeared to warrant further study. There are numerous references in the literature (See Groenewald *et al*, I, 1941) to the rôle played by obesity or condition in the aetiology of Pregnancy Disease, it being generally agreed that heavily conditioned sheep are more susceptible. In the previous experiments the sheep were suddenly switched from an adequately balanced diet to one of poor quality veld hay. This hay was not only deficient in calorific value but also in all the important food constituents, including vitamins. It is, therefore, impossible to say whether any one food fraction is pre-eminently important in the prevention of pregnancy disease or not. In order to test out some of these points the following experiment was carried out.

EXPERIMENTAL PROCEDURE.

Nine non-pregnant, 4 to 6 tooth merino ewes were used. They were in fair condition, being taken from the available sheep on the station. At the beginning of the experiment the sheep varied in weight from 69 lb. to 84 lb. with an average of 79 lb. Their condition can be gauged from the fact that exactly similar non-pregnant ewes used in the previous experiment, when fattened up prior to starvation, averaged 103 lb. in weight.

The sheep were then divided into three equal groups as follows:—

Group 1.—To receive *ad lib.* a mixture of 90 per cent. yellow maize and 10 per cent. meat and bonemeal together with dry veld hay *ad lib.*

Group 2.—As for Group 1 but white maize substituted for yellow.

Group 3.—To receive veld hay only from the beginning.

The sheep were fed in individual feeding boxes and were kept on these rations for 168 days, when Groups 1 and 2 were switched to dry veld hay only (semi-starvation). Blood counts and records of body weights were taken throughout the experiment.

Two other non-pregnant ewes, of the same type but which had been fattened on the same ration as given to Group 1 for some time and weighed 136 and 137 lb. respectively were also used. These also had their ration suddenly cut to dry hay only and were in addition placed in a cold room at 47° F. for 34 days when they were placed back in the camp with the others. The object of the cold room was to increase the carbohydrate metabolism and to accelerate the exhaustion of carbohydrate stored in the body. The results of this second experiment cannot be strictly compared with those of the first, as the cold treatment was only applied in one case, but it is convenient to report the results here for the sake of comparison.

Results.

A. *Body Weight.*

As will be seen from Graph I, Groups 1 and 2 gained weight when put on the experimental ration but Group 1 (yellow maize) increased in weight more rapidly than Group 2 (white maize). After 133 days there was a statistically significant difference between the average weights of the two groups and this difference was maintained till the 168th day when both groups were put on to dry veld hay only. After this change both groups lost weight equally rapidly. Group 3, which was given dry veld hay only from the beginning of the experiment (day 0 on the graph) lost weight at a rate about equal to that shown by Groups 1 and 2. This loss, however, only continued for about 20 days and then became very much more gradual. It will also be noticed that as the average weights of Groups 1 and 2 decreased, so did the rate of loss decrease.

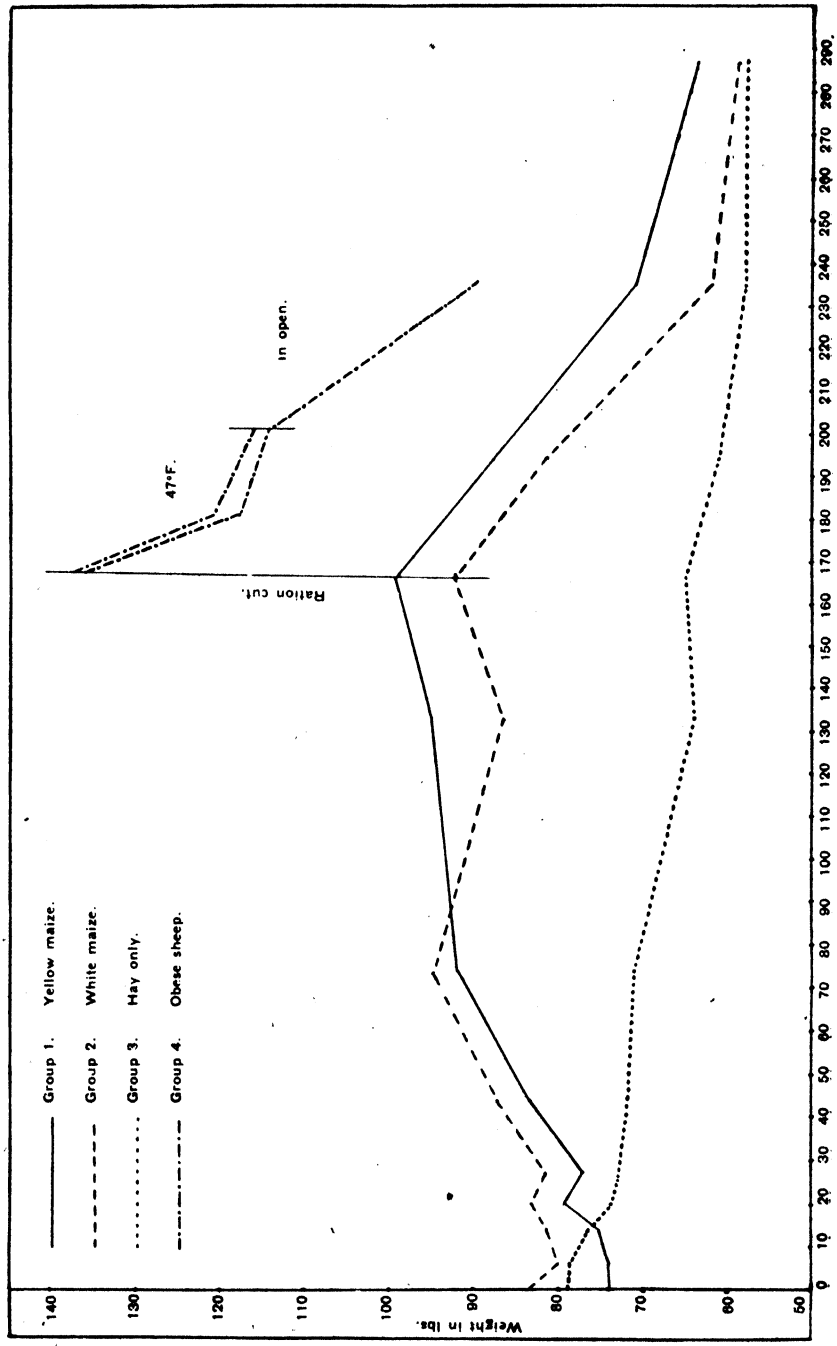
The two sheep that were placed in the cool room lost an average of 22 lb. each in the 34 days they were kept in the room. When placed back with the others but kept on the same ration the loss in weight was 23 lb. in 34 days. The temperature of 47° F., therefore, had no effect on the rate of loss of weight. The average rate of loss is shown in Table 1.

TABLE 1.

Group.	Initial Weight.	Final Weight.	Loss. (lb.).	Time. (Days).	Loss. lb./day.
Cold room..........................	136	90	46	68	0·67
1..........................	99	64	35	119	0·29
2..........................	92	59	33	119	0·28
3..........................	79	58	21	287	0·07

It will, therefore, be seen that the higher the condition of the sheep, the greater the rate of loss of weight.

GRAPH I.—Body Weight.



Reaction to Loss in Weight.

Both the heavy sheep (136 and 137) died suddenly, 59 and 69 days after the cut in the ration.

All the sheep in Groups 1 and 2 survived the 119 days on veld hay only.

One sheep in Group 3 was killed for post-mortem examination after 173 days on veld hay only and another was killed *in extremis* after 214 days. The third survived for 287 days and was discharged.

It has been shown (Groenewald *et al.*, II, 1941) that when 9 non-pregnant sheep of similar class and averaging 111 lb. body weight were put on to a diet of veld hay only, 4 died within a period of 40 days.

B. *The Blood.*

The sheep were bled at weekly intervals and the blood was centrifuged and the red cell precipitate recorded. Total leucocyte counts and differential counts were done and from these figures the total number of neutrophiles and lymphocytes per c.mm. were calculated. There was no significant variation in the other leucocyte types and their totals are not recorded here.

The Erythrocytes.

The percentage red cell precipitate readings are given in Table 2.

TABLE 2.

Effect of Reduced Diet on Red Cell Precipitate Percentage.

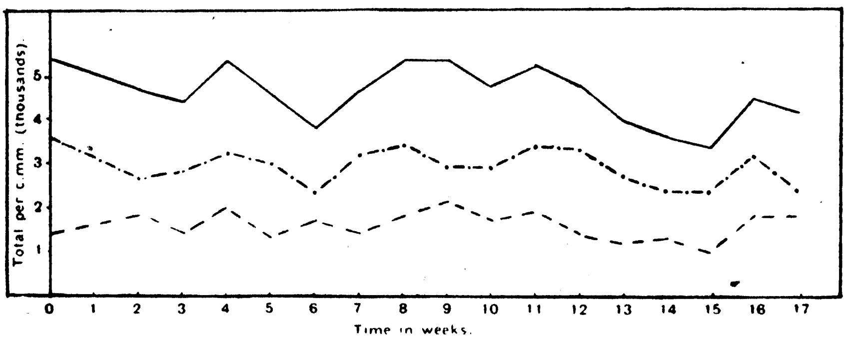
Group.	Condition.	Time in Weeks after Diet Reduced.																			
		2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36	38	40
1	Good	37	36	32	31	29	28	26	25	—	—	—	—	—	—	—	—	—	—	—	—
2	Good	35	32	29	26	26	24	21	21	—	—	—	—	—	—	—	—	—	—	—	—
3	Moderate	38	42	43	—	—	—	—	—	—	—	—	25	22	23	21	—	—	—	—	—
		38	39	40	—	—	—	—	—	—	—	—	25	23	18	18	18	20	21	20	18
		35	40	39	—	—	—	—	—	—	—	—	25	—	—	—	—	—	—	—	—
4	Obese	37	37	33	29	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

The figures for Groups 1 and 2 and the "cold room" (Group 4) experiment are given as averages of the group, but as the sheep in Group 3 died at different periods throughout the experiment their figures are given individually.

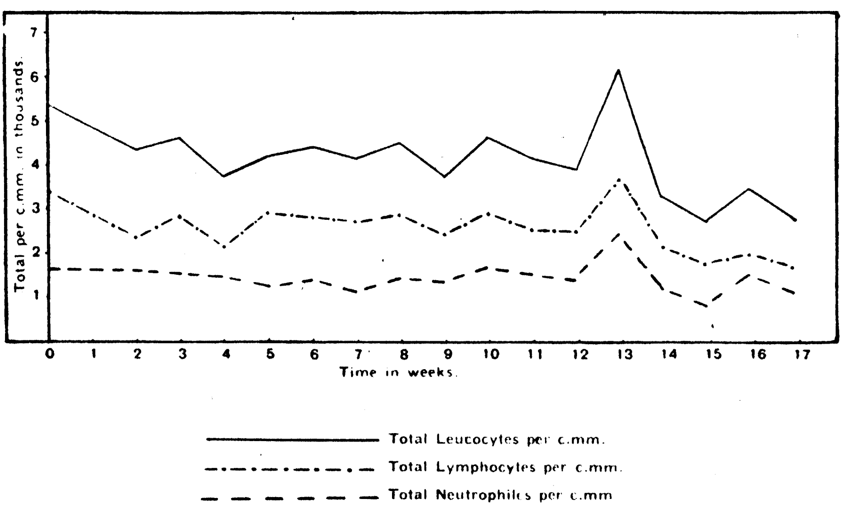
All groups showed a statistically significant drop in percentage erythrocytes on the poor diet, as would be expected, but it is of interest to note that Group 3, the sheep in poorest condition, maintained their red cells better than any other group. After six weeks on veld hay Groups 1, 2 and 4 all showed a drop but every sheep in Group 3 actually showed a rise. Comparing Groups 1 and 2 we see that the yellow maize-fed group maintained their red cells at a consistently higher level than did the white maize group (2). This is apparently to be ascribed to the reserve of vitamin A.

The figures for the leucocytes are given in Graphs II-VI. It will be seen that Groups 1, 2 and 3 all showed a drop in total circulating leucocytes during the period on poor diet. This decline is almost entirely due to a decrease in the lymphocytes, the neutrophiles maintaining their level. This drop in lympohcytes was found to be statistically significant for a combination of all groups and for Groups 2 and 3 individually, but not for Group 1. It would appear that the stored vitamin A also plays a role in maintaining the lymphocyte output. The actual drop in lymphocytes causes a relative neutrophilia, expressed as per cent., but there is no actual rise in neutrophiles.

GRAPH II.—Average Leucocyte Totals. Group I.

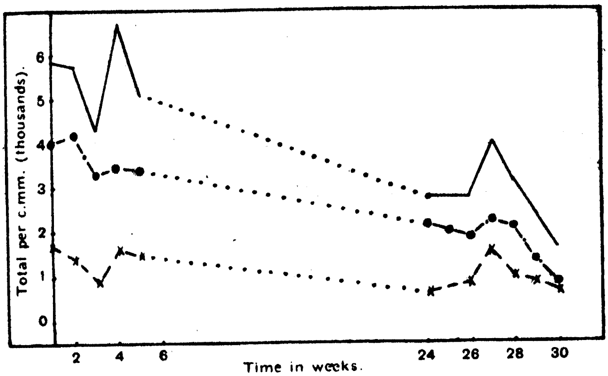


GRAPH III.—Average Leucocyte Totals. Group II.

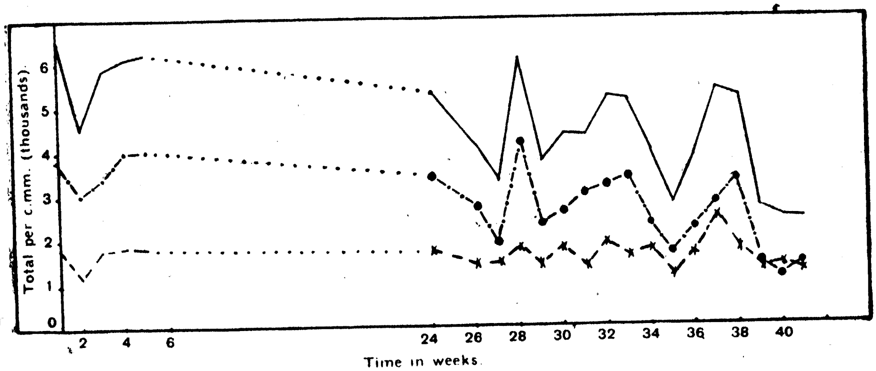


Group 4, the very obese sheep, showed an entirely different picture, namely a drop in lymphocytes and a rise in the neutrophiles, causing an inversion of the normal percentages of these elements, without the drop in leucocytes.

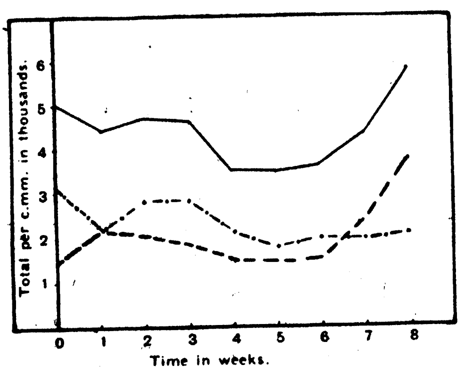
GRAPH IV.—Leucocyte Totals. Sheep A. Group III.



GRAPH V.—Leucocyte Totals. Sheep B. Group III.



GRAPH VI.—Average Leucocyte Totals. Group IV.



Total Leucocytes per c.mm.
Total Lymphocytes per c.mm.
Total Neutrophiles per c.mm.

Post-mortem Findings.

Sheep 1. (*Group* 1.)

Killed at the termination of the experiment.

Adipose tissue.—Fair amount of normal fat still present in the carcass.

Liver, kidney and myocard.—Normal.

Prescapula, Precural and Mediastinal Lymph Nodes.—Desquamation of the littoral cells of the sinuses and formation of foam cells from swollen reticulum cells in the cortex.

Sheep 3. (*Group* 1.)

Killed at the termination of the experiment.

Adipose tissue.—Large amount of fat still present, the abdominal fat showing fat necrosis.

Liver.—Fair amount of fatty infiltration affecting the area round the central veins.

Myocard.—Normal.

Kidney.—Well marked fatty changes of the epithelium of the spiral tubules.

Mediastinal, Mesenteric, Prescapular and Precrural Lymph Nodes.—As in Sheep 1.

Sheep 4. (*Group* 2.)

Killed at the termination of the experiment.

Adipose tissue.—Small amount of fat present. The internal fat shows gelatinous infiltration.

Liver, kidney and myocard.—Normal.

Mediastinal, Mesenteric, Prescapular and Precrural Lymph Nodes.—As in Sheep 1.

Sheep 6. (*Group* 2.)

Killed at the termination of the experiment.

Adipose tissue.—Fair amount of normal fat present.

Liver, kidney and myocard.—Normal.

Mediastinal, Prescapular, and Precrural Lymph nodes.—As in Sheep 1.

Sheep 9. (*Group* 3.)

Killed for examination after 173 days on dry veld hay.

Adipose tissue.—Very little fat present.

All other organs.—Normal.

Sheep 7. (*Group* 3.)

Killed *in extremis* after 214 days on veld hay only.

Adipose tissue.—Gelatinous degeneration of all adipose tissue.

Other organs.—All internal organs showed marked atrophy, ascites and hydrothorax present. Organs otherwise normal.

Precrural and Prescapular Lymph Nodes showed mobilisation of the reticulo-endothelial system as in the other sheep.

Sheep 10. (*Group* 4.)

Died suddenly after 59 days on dry veld hay only.

Adipose tissue.—Large amounts of fat in all the depots showing marked fat necrosis.

Liver.—Gross fatty infiltration of the whole lobule.

Kidney.—Gross fatty changes of the spiral and proximal convoluted tubules. Well-marked desquamation of the tubular epithelium.

Myocard.—Well-marked fatty changes.

Lungs.—Oedema.

Prescapular and Precrural Lymph Nodes.—Disappearance of follicles and well-marked reticulo-endothelial proliferation.

Sheep 11. (*Group* 4.)

Died suddenly after 69 days on dry veld hay only.

Adipose tissue.—Very large amount of fat in the depots showing fat necrosis.

Liver.—Slight central fatty infiltration.

Kidneys.—Well-marked fatty changes in the spiral tubules.

Myocard.—Fair amount of fatty changes

Precrural and Prescapular Lymph Nodes.—Follicles reduced in size and reticulo-endothelial proliferation.

Discussion.

Comparing groups 1 and 2 in the initial (feeding) part of the experiment it is noted that yellow maize was superior to white maize in causing gain in weight. The yellow maize group also maintained the number of both erythrocytes and lymphocytes in the circulating blood, when put on to dry veld hay only, better than did the white maize group. These differences can probably be ascribed to the carotene content of the yellow maize.

Considering the effect of obesity on the reaction to semi-starvation groups 1 and 2 can be combined, the difference between the average weights of these groups being very small as compared with groups 3 and 4. The experiment can, therefore, be considered as follows:—

Very obese sheep.—Group 4.

Good conditioned sheep.—Groups 1 and 2.

Fair conditioned sheep.—Group 3.

All these sheep were non-pregnant ewes of similar type and age, so that the differences in weight can be ascribed to condition, chiefly to extra fat. It will be seen that the sheep reacted in very different ways to a sudden reduction in diet, according to their initial weight.

Very obese sheep.—These two sheep succumbed 59 and 69 days respectively after being put on poor diet. Prior to death they showed hypoglycaemia and acetonaemia as the following table shows.

TABLE 3.

Chemical Analysis of Blood and percentage Lymphocytes in Obese Sheep.

Time in Days.	SHEEP 10.				SHEEP 11.			
	N.P.N.	Sugar.	Acetone.	Lymphocytes.	N.P.N.	Sugar.	Acetone.	Lymphocytes.
				Per cent.				Per cent.
0	31·0	47·2	2·0	61	26·0	45·0	2·0	64
35	25·4	58·2	5·1	44	23·5	58·2	5·7	68
43	30·0	45·5	17·6	50	25·9	43·9	33·5	—
47	—	—	—	60	—	—	—	29
49	—	—	—	—	33·3	29·2	47·0	—
52	30·0	46·3	14·5	—	25·2	44·6	33·7	—
54	—	—	—	38	—	—	—	31
56	—	—	—	—	100·0	111·1	3·7	
59	—	—	—	—	—	—	—	6
63	57·8	36·8	13·9	22		Died 59th day.		
65	85·7	50·0	7·4	—	—	—	—	—
	Died 69th day.							

The sheep showed a typical hypoglycaemia and acetonaemia followed by a rise in N.P.N. and blood sugar and fall in acetone shortly before death. This latter phenomenon has often been encountered in the previous experiments on domsiekte (Groenewald *et al*, 1941) and is considered to be evidence of a sudden increased catabolism of protein for the manufacture of carbohydrates. The sheep died suddenly without symptoms being noted, but the post-mortem findings were typical of pregnancy disease or domsiekte. These sheep can, therefore, be taken as further examples of typical "pregnancy disease" in obese non-pregnant ewes as previously reported (Groenewald *et al*, 1941).

These sheep also showed the typical fall in circulating lymphocytes and rise in neutrophiles which the author has reported in all cases of typical pregnancy disease (Groenewald *et al*, 1941). Hansheimer (1930) has shown that in acidosis of rabbits, caused by inhalation of excess CO_2 or by injection of acids, there is a similar change in the blood picture. Taking the figures for the individual sheep we find that the development of acetonaemia and the drop in percentage lymphocytes roughly correspond. This change may, therefore, be due to the acidosis.

The good conditioned sheep, groups 1 and 2, showed a rate of loss of weight intermediary between that shown between groups 4 and 3. They survived this period of rapid loss and towards the end of the period of semi-starvation their rate of loss had decreased. Out of four slaughtered at the end of 119 days on veld hay, only one showed a very slight fatty liver and

slight fat necrosis. The blood picture is also entirely different from that in pregnancy diseases, being a gradual steady decrease in lymphocytes with no change in the number of circulating neutrophiles.

The sheep in moderate condition (group 3) showed a slow loss of weight, together with the decrease in lymphocytes as seen in groups 2 and 3.

The reaction of sheep to a sudden drop in food intake, therefore, depends largely on the amount of fat present in the body. If there is an initial excess of fat the typical picture of pregnancy disease supervenes with a high acetonaemia, the sheep dying with masses of fat still present in the depôts. The less fat present, the more likelihood there is of sheep surviving the drop in weight and then surviving for a long period on a very poor diet until it eventually dies of cachexia. It is surprising that one sheep of group 3 could survive for 287 days on very poor quality dry veld grass alone. This was probably only possible owing to the fact that the sheep were free of internal parasites and that their water and food were at hand. As the veld hay used in this experiment is similar to what sheep on the highveld of South Africa have to exist on in the winter, it may be concluded that sheep can survive on this very poor feed for amazingly long periods, provided they are kept clear of internal parasites and the energy expended in seeking food and water is curtailed. Stacks of dry veld hay in close proximity to drinking water would, therefore, apparently suffice to save many sheep over our drought periods.

In 1941 Groenewald *et al* stated that, owing to the fact that typical " pregnancy disease " could be produced in non-pregnant ewes, they considered pregnancy only as a contributing factor in the causation of the disease. The present experiment shows that obesity is another potent factor. Why we do not get domsiekte on the grass lands of South Africa has long been a puzzle. The explanation may be that our grassveld sheep never attain that high condition seen in Karroo sheep.

Conclusions.

1. Obesity is a potent factor in the causation of " pregnancy disease " or domsiekte.

2. In the absence of fatal acetonaemia, prolonged semi-starvation of sheep causes a gradual decrease in the circulating lymphocytes without affecting the neutrophiles.

LITERATURE.

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Pigment Metabolism I.—The Examination of the Urine and Blood of Dogs for Bilirubin and Haemoglobin.

By G. C. S. ROETS, Section of Chemical Pathology, Onderstepoort.

In the past the examination of animal diseases in South Africa in relation to the haemoglobin metabolism and disintegration has not been carried out to any great extent. In dogs, especially, no work of this nature has been undertaken. A number of investigations on and routine examinations of biological material have recently been commenced at this Institute. The object of this article is to report on the various examinations already performed on the bilirubin and haemoglobin content of urine and blood of dogs admitted for the purpose of clinical examination. Most of the cases for examination were selected by means of the v. d. Bergh test on the urine which is a routine clinical examination.

When bilirubin is detected in the urine, the routine procedure is to examine the plasma bilirubin. This latter examination is all the more necessary as a number of cases of bilirubinuria have been shown to be due to hepatic derangement. Full reliance cannot be placed on the microscopic examination of smears or on the symptomatology for the diagnosis of canine piroplasmosis as an occasional case shows negative smears notwithstanding a careful examination and practically no symptoms of the disease.

The value of the examination of the urine and especially of the plasma for the presence of bilirubin and haemoglobin has proved to be such that the continuation and expansion of this method of diagnosis are indicated.

Literature.

In a review on jaundice McNee (1923) refers to the observations of Virchow (1847) who believed that haemotoidin was an isomer of bilirubin and was responsible for haemolytic jaundice and to those of Minkowski and Naunyn (1886) who found that geese whose livers had been surgically removed did not show jaundice when poisoned by arsine. Virchow's claim that there were two types of bilirubin, namely, one present in cases of hepatic disturbances and the other in haemolytic jaundice, was neglected for many years on account of the findings of Minkowski and Naunyn. McNee (1912), however, repeated the experiments of Minkowski and Naunyn and determined that the removal of the liver in birds did not produce the same effect as the removal of that organ in higher animals. In birds the reticulo-endothelial cells (Kupffer cells) are mainly concentrated in the liver, whereas in higher animals, such as dogs, these cells also occur in other parts of the body [Aschoff (1922)]. Prusik and McNee (1924) demonstrated the formation of bilirubin outside the liver.

Blankenhorn (1921) dialyzed icteric plasma and demonstrated dialyzable and non-dialyzable pigments. He expressed the opinion that non-dialyzable pigment was associated with the blood proteins, Andrews (1924) on the other hand presented experiments which indicated that the linkage of bilirubin with protein or with any phospholipoid probably did not occur.

V. d. Bergh (1918) introduced a method of differentiation between the two types of bilirubin. This method is even at present commonly empolyed and is based on the principle of the Ehrlich's diazo-reaction.

Collison and Fowweather (1926) found that free bilirubin isolated from heavily pigmented gallstones, behaved like the bilirubin present in cases of haemolytic jaundice when tested by the v.d. Bergh method, i.e., it gave a positive indirect reaction. The alkaline salt of bilirubin, however, behaved like the bilirubin found in cases of hepatic disturbances, i.e., it gave a positive direct v. .d. Bergh reaction. They expressed the view that bilirubin in serum giving the direct reaction was probably the ammonium salt of the free acid. The ammonium is probably derived from amino-acids in the liver cells during the course of urea production. Subsequently various workers stressed the importance of the pH of solutions on the v. d. Bergh reaction [Davies and Dodds (1927), M'Gowan (1929), Elton (1931)].

The differentiation of the two types of bilirubin found in icteric sera is of great value in establishing whether an icterus is due to hepatic disturbance or to haemolysis [McNee (1924), v. d. Bergh (1924), Willcox 1924), Elton (1931), etc.]. The hepatic and haemolytic types were called by Elton (1931) the crystalloid and suspensoid colloid respectively.

Technique.

1. *Bilirubin.*—A. *Plasma Bilirubin.*

The technique employed for the estimation of plasma bilirubin is the method of v. d. Bergh (1918), improved by v. d. Bergh and Grotepass (1934).

Reagents.

I. *Solution* A.—1 gm. sulphanilic acid, 15 c.c. of 25 per cent. HCl, and distilled water up to 1 litre.

II. *Solution B.*—0·5 per cent. sodium nitrite in aqueous solution.

III. *Reagent.*—The reagent is prepared by adding 10 c.c. of solution A to 0·3 c.c. of solution B. This solution must be freshly prepared before use.

IV. *Buffer Solution.*—27·25 c.c. of 0·1 Mol. citric acid and 72·75 c.c. of 0·2 Mol. secondary sodium phosphate (the pH of such a solution is 6·6).

V. *Buffer Alcohol.*—50 per cent. alcohol containing 10 c.c. of the buffer solution per 100 c.c.

(a) Direct Reaction.

For the quantitative estimation of bilirubin in plasma giving the direct reaction, a mixture of 2 c.c. reagent, 1 c.c. plasma and 2 c.c. water is diluted up to 10 c.c. (or more in cases of high concentrations of bilirubin) with the buffer alcohol solution.

(*b*) *Indirect Reaction.*

For the quantitative estimation of bilirubin in plasma giving the indirect reaction, the plasma proteins are precipitated by mixing one part of plasma with two parts of 96 per cent. alcohol. The mixture is centrifuged and to 8 c.c. of the supernatant fluid 2 c.c. of the reagent is added.

Comparisons.—The comparisons are made in a colorimeter against a known standard. V. d. Bergh and Grotepass used smoked glass or painted wire gauze as standards for comparison. Because these standards were not available a number of solutions used by various workers were investigated as possible substitutes.

The various solutions tested were:—

I. 2·16 per cent. cobalt sulphate [McNee (1925)].

II. The ethereal rhodate iron solution [Hawk and Bergeim (1931)].

III. Standard methyl red solution, pH 4·63 [Beaumont and Dodds (1939)].

IV. 10 per cent. cobalt nitrate [Wester (1935)].

V. A bilirubin standard containing 5 mg. bilirubin per litre [v. d. Bergh and Grotepass (1934)].

These standards were compared against one another in a colorimeter but no two were found to match. The following experiments were, therefore, made:—A solution of bilirubin, containing 5 gm. bilirubin per 800 c.c. was made as described by v. d. Bergh and Grotepass (1934). A mixture of 8 c.c. of this solution and 2 c.c. of the reagent was used in each experiment. Immediately after the reagent had been added the solution was transferred to a colorimeter cup and observed in comparison with the 10 per cent. cobalt nitrate standard (fixed at a depth of 30 mm.). There occurred a gradual change in the colour of the reagent mixture. It was only after two minutes that a good match of the colours was obtained at 38 mm. After 3 to 4 minutes the best match was obtained at 32-30 mm. After 4 minutes it was no longer possible to match the colours. In an attempt to find a stage at which the colour of such a reacting mixture would remain constant, the colours of a series of such mixtures which had been allowed to react in a dark cool place for 15 minutes and for 4, 6, 24 and 48 hours were examined. No constant stage was, however, obtained. The best match was also obtained after 3 to 4 minutes when plasma or urinary bilirubin was used instead of the pure bilirubin solutions. Consequently the amount of bilirubin in materials under examination could be calculated from the results obtained in comparison with a standard of 10 per cent. cobalt nitrate solution equivalent to a mixture containing 5 mg. bilirubin per 1000 c.c. (equivalent to 1 v. d. Bergh unit).

Differentiation of the Direct and the Indirect Types of Bilirubin.

Lepehne's technique [McNee and Keefer (1925)] was employed to determine whether the plasma bilirubin gave the direct, indirect or delayed v. d. Bergh reaction. In three tubes were placed 2·0 plasma containing the bilirubin. To tube 1 was added 0·2 c.c. of water. This was the negative control. To tube 2 was added a small amount of caffeine-sodium salicylate. When this had dissolved, 0·2 c.c. of reagent was added. The reaction commenced immediately, but time should be allowed for it to reach its maximum. To

tube 3 the reagent (0·2 c.c.) is now added. The response can then be compared with that obtained in tube 2, i.e., the direct or delayed reaction could be judged by the intensity of the colour and its rapidity of development.

B. *Bilirubin in Urine.*

Methods Considered.

Marsh (1940) dissolved dried egg albumen up to 10 per cent. in strongly icteric urines. He then applied the indirect v. d. Bergh method for estimating the bilirubin quantitatively. Hunter (1930) absorbed the urinary bilirubin with barium chloride and then recovered it from the absorbent, which was filtered off, by dissolving it in 96 per cent. alcohol.

The bilirubin present in urines of cases examined at this laboratory always was the direct type. Because bilirubin of the direct type is only sparingly soluble in alcohol concentrations of 60 per cent. and higher, there occurred appreciable losses of bilirubin, when the above-mentioned methods were used. It was also observed that all the bilirubin present in urine was not absorbed by the barium chloride.

The method of Siede and Zink (1931), based on the number of drops of a 2 per cent. aqueous solution of methylblue necessary to produce a green colour in a known volume of urine is even less satisfactory, especially as the colour of icteric urines makes it impossible to judge the endpoint.

Method Employed.

The direct v. d. Bergh technique was employed to get an idea of the concentration of bilirubin in urine. Quantitative estimations could not be made in urines coloured by pigments such as haemoglobin. The quantity of haemoglobin in the urine of a dog suffering from piroplasmosis was estimated at 498 mg. per 100 c.c. urine. The haemoglobin was precipitated by alcohol using 1 part of urine and 4 parts of alcohol, and centrifuged. The supernatant liquid was fairly clear. The bilirubin in this solution was found to be 20 v. d. Bergh units. It was, however, felt that such a figure was not justifiable because the direct type of bilirubin, which was the one present, is only very sparingly soluble in alcohol of such a high concentration.

C. *Haemoglobin.*

The haemoglobin determinations were made by the method developed by Roets (1940). The haemoglobin was converted into pyridine haemochromogen and the intensity of the 555 spectroscopic absorption band measured against that of a standard prepared from pure haemin.

Details of Materials Examined.

Blood.—The blood for the examination was in every case drawn from the jugular vein, the anticoagulent used being 0·5 c.c. of a 20 per cent. potassium oxalate solution per 25 c.c. of blood.

Urine.—The urine was collected in a beaker during the act of urination.

Both urine and blood samples were examined immediately after collections were made.

Results.

In the tabulated data, quantities of bilirubin less than one v. d. Bergh unit are expressed as "positive". If haemoglobin be also present the bilirubin is expressed as "positive +".

The plasma bilirubin and haemoglobin concentrations in bloods of various cases examined are presented in Table 1.

Table 1.—*Bilirubin and Haemoglobin in Blood.*

Date.	No. of Dog.	Disease.	Haemoglobin in gm. per 100 c.c.	Bilirubin.	
				Type.	v. d. Bergh Units.
5/ 8/39	253/39	Hepatic disturbance.......	10·66	Direct.......	5
16/ 8/39	261/39	Piroplasmosis..............	6·99	Indirect.....	1·25
13/10/39	321/39	Piroplasmosis..............	3·0	Indirect.....	Positive.
17/ 1/40	17/40	Normal....................	16·2	Negative.....	Negative.
8/ 2/40	37/40	Piroplasmosis..............	9·34	Indirect.....	Positive.
14/ 2/40	41/40	Piroplasmosis..............	11·9	Indirect.....	Positive.
28/ 3/40	99/40	Piroplasmosis..............	3·7	Indirect.....	53
13/ 4/40	118/40	Rheumatism..............	13·7	Negative.....	Negative.
18/ 7/40	182/40	Piroplasmosis..............	9·0	Indirect.....	3
5/ 9/40	238/40	Enteritis..................	14·4	Indirect.....	Positive.
14/ 2/41	71/41	Hepatic disturbance.......	17·8	Direct.......	3
14/ 3/41	71/41	Hepatic disturbance.......	14·0	Direct.......	2·5
27/ 3/41	71/41	Hepatic disturbance.......	17·9	Direct.......	3·2
19/ 4/41	205/41	Piroplasmosis (Chr.)........	14·4	Indirect.....	Positive.
20/11/41	441/41	Piroplasmosis..............	7·8	Direct delayed	2·2

Results obtained on bilirubinuria and haemoglobinuria are summarized in Table 2.

Table 2.—*Bilirubin and Haemoglobin in Urine.*

Date.	No. of Dog.	Disease.	Bilirubin in v. d. Bergh Units.	Haemoglobin in mg. per 100 c.c.
5/ 8/39	253/39	Hepatic disturbance..................	3	Negative.
16/ 8/39	261/39	Piroplasmosis.........................	10	4
11/10/39	304/39	Normal...............................	Negative.....	Negative.
4/11/39	329/39	Piroplasmosis.........................	Positive.....	Negative.
5/ 1/40	4/40	Piroplasmosis.........................	1·9	Negative.
13/ 1/40	7/40	Piroplasmosis.........................	Positive +..	12·83
17/ 1/40	17/40	Normal...............................	Negative.....	Negative.
14/ 2/40	41/40	Piroplasmosis.........................	Positive.....	Negative.
22/ 2/40	48/40	Piroplasmosis.........................	Positive +..	99·7
27/ 3/40	97/40	Piroplasmosis.........................	Positive +..	799
28/ 3/40	99/40	Piroplasmosis.........................	Positive +..	498
13/ 4/40	118/40	Rheumatism.........................	Negative.....	Negative.
18/ 7/40	182/40	Piroplasmosis.........................	5·6	Negative.
14/ 3/41	71/41	Hepatic disturbance..................	15	Negative.
27/ 3/41	71/41	Hepatic disturbance..................	7·5	Negative.
20/11/41	441/41	Piroplasmosis.........................	Positive.....	Negative.

In some cases simultaneous examinations of both urine and blood were made for haemoglobin and bilirubin. These results are presented in Table 3.

TABLE 3.

Date.	No. of Dog.	Disease.	Blood.			Urine.	
			Haemoglobin in g. per 100 c.c.	Plasma Bilirubin.		Haemoglobin in mg. per 100 c.c.	Bilirubin in v. d. Bergh Units.
				Type.	Amount in v. d. Bergh Units.		
5/ 8/39	253/39	Hepatic disturbance	10·66	Direct...	5	Negative	3
16/ 8/39	261/39	Piroplasmosis.......	6·99	Indirect.	1·25	4	10
11/10/39	304/39	Normal............	10·4	Negative	Negative	Negative	Negative.
14/ 2/40	41/40	Piroplasmosis.......	11·9	Indirect.	Positive.	Negative	Positive.
28/ 3/40	99/40	Piroplasmosis.......	3·7	Indirect.	53	498	Positive+
18/ 7/40	182/40	Hepatic disturbance	9	Indirect.	3	Negative	5·6
14/ 3/41	71/41	Hepatic disturbance	14	Direct...	2·5	Negative	15
27/ 3/41	71/41	Hepatic disturbance	17·9	Direct...	3·2	Negative	7·5
16/ 6/41	205/41	Piroplasmosis (Chr.)	14·4	Indirect.	Positive.	4	Positive.
20/11/41	441/41	Piroplasmosis.......	7·8	Direct delayed	2·2	Negative	Positive.

DISCUSSION.

To interpret the results in cases of bilirubinaemia and bilirubinuria, considerations should be given to the origin and the fate of bilirubin. Watson (1938) deals with this problem. After haemoglobin is transformed via haematin into bilirubin by the reticulo-endothelial system [Aschoff (1922)], it passes to the liver and, under normal conditions, is excreted via the bile duct into the intestinal tract, where it is broken down into products such as urobilinogen and stercobilinogen. A portion is passed out with the faeces and a portion is reabsorbed and excreted in the urine.

In diseases such as piroplasmosis the abnormal destruction of erythrocytes and the resultant production of bilirubin produce a rise in plasma bilirubin. Bilirubin under such conditions is partly absorbed by tissues such as fats or mucous membranes and partly excreted. By such excretions the bilirubin concentrations of urines, which is normally very low [v. d. Bergh (1924)], are considerably increased.

In the case of hepatic disturbances the bilirubin generated in normal or abnormal amounts, passes from the liver into the circulation and thus the level of plasma bilirubin is raised. This eventually gives rise to absorption by various tissues and an increased bilirubin excretion in the urine.

When the results summarised in Table 1 are considered, the cases examined can be grouped according to whether the plasma bilirubin gives the indirect or direct v. d. Bergh reaction, into haemolytic and hepatic disturbances cases respectively, with the one exception of case No. 441/41, suffering from piroplasmosis. In this particular case the examination was made after all the parasites had disappeared from the blood. The bilirubinaemia, however, was still present. Finally liver damage probably due to protozoal intoxication [Willcox (1924)] occurred.

Haemoglobinuria frequently occurs in canine piroplasmosis. In such cases the haemoglobin disintegration in the body is not sufficiently rapid to cope with all the haemoglobin liberated by the destruction of the erythrocytes, consequently the unchanged haemoglobin is excreted in the urine. This is especially evident in acute cases, such as Nos. 99/40 and 97/40, in which bilirubin as well as free haemoglobin are excreted in the urine. The process of haemolysis was definitely interferred with in the case of No. 441/41 by the treatment, which was probably the cause of the absence of haemoglobin in the urine. The absence of haemoglobin in the urine of cases such as Nos. 182/40, 41/40, 4/40 and 329/39, may be due to various factors, such as mild infections or different stages of the course of the disease. Such factors will receive due consideration during the course of investigations to be made in the near future.

The haemoglobin concentrations in the blood of the piroplasmosis cases (Tables 1 and 3), as would be expected, are low except in the chronic case 205/41. In this case the haemoglobin destruction was probably at a very low rate. In the case of hepatic disturbances, however, the figures for the haemoglobin are similar to those of normal dogs.

Summary.

1. In all the cases of bilirubinaemia examined, whether the animals suffered from canine piroplasmosis or hepatic disturbances abnormal amounts of bilirubin were excreted in the urines.

2. Haemoglobin was frequently present in the urine of dogs suffering from piroplasmosis. The highest concentration of haemoglobin found was 799 mg. per 100 c.c. urine. No haemoglobin could be detected in the urines of dogs suffering from hepatic disturbances.

3. The plasma bilirubin of the piroplasmosis cases gave an indirect v. d. Bergh reaction whereas that of the hepatic disturbance cases gave the direct reaction with the v. d. Bergh reagent. The highest plasma bilirubin figure obtained was 53 v. d. Bergh units in a case of canine piroplasmosis.

4. In the piroplasmosis cases an increase in bilirubin was accompanied by a decrease in haemoglobin in the blood, whereas in the hepatic disturbance cases there was no decrease of the blood haemoglobin, the bilirubin in the plasma being due to retention and not to haemolysis.

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Pigment Metabolism II.—Determinations of Bilirubin and Carotinoids in the Plasma and of Coproporphyrin in the Urine and Faeces of Cattle Experimentally Infected with *Theileria parva*.

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IN *Theileria parva* infection yellow staining of the fats and the mucous membranes is not conspicuous and, in any case, is much less intense than in certain other protozoal diseases such as anaplasmosis. It had, however, been noted that the urine of cases of *Theileria parva* infection was at times pigmented. Prior to these investigations, however, it was generally held that icterus did not occur in *Theileria parva* infection.

The examination of those cases of the disease in which highly coloured urine did occur revealed that abnormal amounts of urobilin and coproporphyrin were present, that the subcutaneous and peritoneal fat contained both bilirubin and carotinoids and that the yellow colour was due to both these pigments.

On account of the above findings it was decided to carry out a more detailed examination of cases experimentally infected, the object of the experiment being to establish whether or not abnormal pigmentation could be established in the urine, plasma, faeces and fats.

The method employed in this experiment was to examine bovines, experimentally infected with *Theileria parva by* ticks, both before and during the course of the disease as to the presence of bilirubin and carotinoids in the plasma and fat and of coproporphyrin in the urine and faeces.

LITERATURE.

1. *Bilirubin and carotinoids in plasma and fat.*

A review of the literature on bilirubinaemia and bilirubinuria was made in number 1 of the series of these communications (Roets, 1942). It is known that the plasma of men and animals ingesting large amounts of green plant food contains carotinoids. Thus a "pseudo-icterus" can be produced in man feeding for a few weeks large amounts of green vegetables [v. d. Bergh (1924)]. Rimington (1938) estimated quantitatively the bilirubin and carotinoids in icteric bovine plasma and discussed the respective contribution of the two pigments towards the colouration of such plasma.

The intensity of the yellow colour (icterus index) produced by bilirubin is not always in direct proportion to the amount of bilirubin in the different plasmas [Elton (1931)].

Rimington and Fourie (1938) introduced a method of differentiating carotinoids and bilirubin in fats.

II. *Coproporphyrin in Faeces and Urine of Cattle.*

Rimington and Roets (1937) found not only coproporphyrin I but also coproporphyrin III in the faeces and the urine of bovine congenital porphyrinuria cases. Quantitative determinations of these coproporphyrin isomers were reported upon by Rimington, Roets and Fourie (1938). The presence of these isomers in the faeces and the urine of bovines infected with *Theileria parva* was first described by Roets (1938). The excretion of coproporphyrin in the faeces and the urine of normal bovines has also been determined [Fourie and Roets (1939); Roets (1941)].

TECHNIQUE.

I. *Bilirubin of Plasma.*

The method employed for the quantitative estimation of plasma bilirubin was that of v. d. Bergh and Grotepass (1934) as described in number I of this series of communications [Roets (1942)].

II. *Carotinoids of Plasma.*

The amount of carotinoids in plasma was determined by the method of Rimington (1937) viz.:—

The carotinoids were precipitated with the plasma proteins by mixing 15 c.c. of plasma with 30 c.c. of 96 per cent. alcohol. After centrifugation the protein mass was extracted two to three times with ether, about 20 c.c. of fresh ether being used for each extraction. The combined ethereal extracts were transferred to a separatory funnel, shaken with 0·1 N.NaOH solution to remove any bilirubin present, and then washed with distilled water to remove the alkali. The ethereal solution was evaporated on a water bath down to 15-50 c.c. (depending on the concentration). The colour intensity of this solution was measured in a colorimeter against a dye standard [Guilbert (1934)] and the amount then calculated.

III. *Differentiation of the Carotinoids and the Bilirubin in Fat.*

The method employed to differentiate carotinoids from bilirubin in fat was originated by Rimington and Fourie (1938). A 2 gm. sample of fat, as free as possible from blood, was boiled in a strong glass test tube with 5 c.c. of 5 per cent. aqueous sodium hydroxide. The tube was cooled rapidly to about blood temperature and then approximately an equal amount of ether was added. The mixture was well shaken and set aside until it

separated into two layers. The bile pigments remained in the lower greenish yellow aqueous layer, the carotinoids in the upper yellowish ethereal layer.

IV. *Coproporphyrin in Urine and Faeces.*

The coproporphyrin was extracted by acetic acid and ether from urine and faeces as described by Fourie and Roets (1939) for those of normal cattle. The coproporphyrin was transferred from the ethereal solution to 5 per cent. hydrochloric acid solution. The intensity of the spectroscopic absorption bands in this solution was measured against that of a standard coproporphyrin solution and the amount then calculated.

The coproporphyrin I to coproporphyrin III ratios were obtained by separating the methyl esters as described by Rimington, Roets and Fourie (1938).

Experimental Details.

All the cases of *Theileria parva* infection were produced by tick infestation.

Bilirubin was first determined in the plasma of two bovines, 7076 and 6371, suffering from the disease. The plasma was examined for bilirubin and carotinoids, fats collected at post mortem for bilirubin and carotinoids and the urine for coproporphyrin.

Two 2 year old bovines (6722, 7031) were infected and the examination was carried out on the plasma for bilirubin and carotinoids and on the faeces and urine for coproporphyrin before the reaction commenced and up to the time of death. The ratio of coproporphyrin I to coproporphyrin III was determined in the faeces of each of these two animals two days before death. The coproporphyrin I to coproporphyin III ratio of the urine was also determined in a sample accumulated over two days subsequent to the period of incubation.

A similar examination to the previous one was carried out on bovines 6725 and 6902 except that the faeces of these were examined as combined samples for the reason that they shared a box.

The blood for the determinations on the plasma was drawn from the jugular. The anticoagulant was 0·75 c.c. of a 20 per cent. potassium oxalate solution per 50 c.c. blood.

The animals were not housed in metabolism boxes as they had to be kept in quarantine on account of the danger of the disease spreading to other animals. The faeces were collected from each animal before the stables were cleaned. The total faeces for each day were thoroughly mixed and samples for analysis were then taken.

The urine was collected in glass jars during the act of urination and was immediately examined.

RESULTS.

(*a*) Quantitative estimations of bilirubin and carotinoids were made from time to time in the plasma of bovines 7076 and 6371 infected with *Theileria parva* on the 3.11.37. Bovine 6371 was killed *in extremis* on 28.11.37 and 7076 died on the 7.12.37. The data obtained are presented in Table 1.

TABLE 1.

No. of Animal.	Date.	Mg. Carotinoids per Litre Plasma.	Plasma Bilirubin in v. d. Bergh Units.
7076	19/11/37	2·9	1·97
7076	22/11/37	3·7	2·02
7076	24/11/37	3·4	1·0
7076	29/11/37	2·04	1·57
6371	19/11/37	3·7	2·02
6371	22/11/37	3·0	4·33
6371	24/11/37	—	4·25
6371	27/11/37	—	5·12

(*b*) Bovines 6722 and 7031 were infected on the 25.4.38. They reacted on the 6.5.38 and the 5.5.38 respectively. The results obtained on their plasma bilirubin and carotinoids and faeces coproporphyrin are presented in Table 2. Plasma bilirubin less than 1 v. d. Bergh unit is expressed as "Positive".

TABLE 2.

Date.	BOVINE 6722.			BOVINE 7031.		
	Faeces.	Plasma.		Faeces.	Plasma.	
	Mg. Copro-p. per 100 g.	v. d. Bergh Units Bilirubin.	Mg. Carotinoids per Litre.	Mg. Copro-p. per 100 g.	v. d. Bergh Units Bilirubin.	Mg. Carotinoids per Litre.
26/4/38	0·018	Trace only	3·6	0·014	Negative	4·6
28/4/38	0·025	Negative	4·1	0·022	Negative	5·0
3/5/38	0·014	Negative	4·5	0·025	Negative	3·7
5/5/38	0·016	—	—	0·038	—	—
7/5/38	0·024	Positive	4·8	0·093	Positive	3·8
9/5/38	0·039	Positive	3·3	0·104	Positive	2·6
10/5/38	0·031	Positive	3·1	0·082	Positive	—
11/5/38	0·094	2·0	—	0·082	Positive	—
12/5/38	—	5	1·5	—	Positive	1·7
13/5/38	0·128	—	—	0·065	—	—
14/5/38	—	1	—	—	Positive	—
16/5/38	0·082	Positive	—	0·091	—	—
17/5/38	0·144	—	—	0·146	—	—
18/5/38	0·102	8·3	—	0·108	7·8	—
20/5/38	—	10·5	0·9	0·271	9	0·4

TABLE 3.

Date.	BOVINE 6725. Plasma Bilirubin in v. d. Bergh Units.	BOVINE 6902. Plasma Bilirubin in v. d. Bergh Units.	Mg. Copro-p. per 100 g. in the combined Faeces of 6725 and 6902.
21/10/38	Negative	Negative	—
24/10/38	—	—	0·04
25/10/38	Negative	Negative	0·055
28/10/38	Negative	Negative	—
31/10/38	—	—	0·06
1/11/38	Positive	Positive	—
2/11/38	—	—	0·075
4/11/38	5·1	3·9	0·12
7/11/38	11·25	3·7	0·182
8/11/38	9·375	5·0	No faeces passed.
9/11/38	9·2	7·03	0·5
10/11/38	11·1	7·7	0·151
11/11/38	10·0	6·8	0·322
14/11/38	12·0	7·3	—

The concentration of coproporphyrin in the urine of 6722 on the 18th and 19th of May was 0·338 and 0·202 mg. per 2000 c.c. respectively. In the urine of 7031 on the 18th of April the coproporphyrin concentration was 0·395 mg. per 2000 c.c. These coproporphyrin fractions of the two animals were combined and the ratio of coproporphyrin I to coproporphyrin III in the combined sample was found to be 1 : 0·31.

The coproporphyrin samples from the faeces of 6722 on the 11th and 13th of April, on which the quantitative estimations of coproporphyrin had been made, were combined. The ratio of coproporphyrin I to coproporphyrin III in this combined sample was 1 : 1·5. In a similar composite sample of coproporphyrin from the faeces of 7031 collected on the 9th, 10th and 11th of April, only a trace of coproporphyrin I was detected.

(c) Bovines 6725 and 6902 were infected on 21.8.38. The results obtained on their plasma bilirubin and on the coproporphyrin concentrations of their combined faeces are presented in Table 3.

Bovine 6725 was killed *in extremis* on the 14th November and 6902 on the 16th November.

Samples * of peritoneal and subcutaneous fats from all the animals examined were collected at post mortem. Carotinoids and bilirubin were found to be present in all the cases.

* A sample of urine collected during the post mortem examination of a typical case (bovine 8593) was sent to my laboratory for examination on the 22nd of January, 1943. This sample contained 0·4 mg. Coproporphyrin per 2000 c.c. urine. This sample of urine was also examined for haemoglobin by the pyridine haemochromogen method [Roets (1940)]. A haemoglobin concentration of 210 mg. per 100 c.c. urine could be determined.

Discussion.

The following points should be borne in mind when considering the significance of the data presented:—

I. The concentration of the carotinoids in the plasma depends on the type of food fed to the animals and is not associated with haemopoietic activities.

II. The concentration of the bilirubin in the plasma in a protozoal disease, such as *Theileria parva* infection, is indicative of haemoglobin disintegration.

III. Coproporphyrin is of endogenous origin and it is excreted via the bile and urine. Thus the relative quantities appearing in the faeces and the urine depend largely upon the total excretion of faeces and of urine.

In the advanced stages of the disease the animals ate little or no food. The intake of food gradually decreased and ultimately ceased and the amount of faeces passed decreased. For example, bovines 6725 and 6902 (Table 3) showed decrease of food intake and passed no faeces on the 18.11.38. The concentration of coproporphyrin in the faeces passed the following day was the highest during the whole period of examination. In all cases examined the amounts of faeces passed during the advanced stages of the disease were very small, whereas the concentrations of coproporphyrin increased considerably in such faeces. It is, however, doubtful whether the total amount of coproporphyrin excreted increased.

The presence of both coproporphyrin I and coproporphyrin III in the faeces and the urine of *Theileria parva* infected is of special interest. Haematin and the bile pigments are type III pigments. The type III pigments are sometimes called the "physiological" types. Theories, such as those of Rimington (1937b) and Turner (1940), were put forward to explain the relationship of natural porphyrins and their dualism to normal and pathological haemopoiesis. These theories are based on the presence of a porphyrin stage in haemoglobin metabolism. The type I porphyrins are considered to be "byproducts" in the process of haemopoiesis or "products" which result from the acceleration of the control processes in the direction of the type I products.

It is known that in normal bovines both coproporphyrin I and coproporphyrin III are excreted in the faeces and urine [Fourie and Roets (1939); Roets (1941)]. The effect of *Theileria parva* infection on the blood forming tissues, therefore, does not prevent the formation of both types. On account of the very small amounts encountered in such investigations it is extremely difficult to determine the influence of the disease on the quantitative relationship of the two types during the course of the disease.

The level of the bilirubin in the plasma of the same animal fluctuated considerably from day to day, especially during the early stages of the disease (Tables 1, 2 and 3), but ultimately in all the animals, shortly before death, it reached a high level. The highest levels obtained in the different animals also differed considerably.

Summary.

The investigations on *Theileria parva* infection of cattle revealed:—

(1) That bilirubinaemia did occur, the highest figure for plasma bilirubin being 12 v. d. Bergh units.

(2) That the yellow staining of the fat is due to the combined effect of carotinoids and bilirubin.

(3) That the intensity of the yellow colour of the plasma is in part due to carotinoids, a normal constituent of the blood of cattle, especially if they be fed on food containing carotinoids.

(4) That there is a definite rise in the concentration of coproporphyrin in the faeces and urine. Such a rise, however, does not necessarily mean that there is an increase in the total amount excreted as the rise may be due to the decrease in the amount of faeces passed as a result of the disease.

Acknowledgment.

I wish to thank Mr. W. O. Neitz, Section of Protozoology and Virus Diseases, Onderstepoort, for supplying the material for examination.

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Genetics in the Diagnosis of Bovine Congenital Porphyrinuria (Pink Tooth).

By P. J. J. FOURIE, Section of Hygiene, Onderstepoort.

THE animal which is the subject of this paper is a Shorthorn bull No. 7597. Fourie (1939) gave a clinical description of the animal. The bull at that time showed acute skin lesions, when the animal is exposed to the sun. (See figure 1).

When the animal is protected against the sun by stabling for instance, there is immediate improvement in the lesions. Unlike Fourie's (1936) other cases and Fourie and Rimington's (1928) Cedara case, there is no discolouration of the teeth and urine. On analysis Roets (1939) found 11·298 mgm. of total daily porphyrins excreted. This is considerably lower than is the case with undoubted bovine porphyrin sufferers. (7016—mild case—·3 gm; 7017 and 7018—severe cases—1·6 gm. and ·8 gm. respectively. This low level of total daily porphyrin excretion in bull 7597 seems nevertheless to be higher than is the case with a completely normal or apparently normal animal (3 mgm. to 8·9 mgm.) (See Fourie and Roets 1939).

In view of the fact that bull 7597 is markedly photosensitive, has a total daily porphyrin excretion on a somewhat higher level than would appear to be the case with normal bovines, and as shown by Fourie (1939) has a common ancestry, although this is admittedly very remote, with the bull Royal Regent, which Fourie (1939) has shown to be the possible transmitter of the porphyrin carrying gene to a South African Shorthorn herd, Fourie (1939) regarded this bull as a suspicious case of bovine congenital porphyrinuria which could really only be confirmed in the living animal genetically by breeding experiments. This bull was, therefore, placed in a camp with cows 7023 [a known clinical case of pink tooth (Fourie 1936, 1939)] and cow 7354 [also a known clinical case of pink tooth, the so-called Cedara case (Fourie and Rimington 1938)]. Unfortunately the bull contracted heartwater in the camp and in spite of treatment with uleron died either from heartwater or the effects of the uleron treatment. Bull 7597 was not actually seen to serve cows 7023 and 7354, but as this was the only bull running with these cows in the camp, it is reasonable to assume that this bull is the sire of the two calves which were in due course born out of cows 7023 and 7354. Both the calves are clinically normal. Fourie (1939) has shown that pink tooth in bovines is inherited as a recessive character. Consequently one would expect that if a bull which is an affected case, is mated to affected cows the progeny born from such mating must all be affected cases. In the above breeding experiment, we know that the two cows are undoubtedly cases of

congenital porphyrinuria. The bull 7597 can, therefore, not be a typical porphyrin sufferer. In other words, if the porphyrin inherited character is a simple mendelian recessive, this bull 7597 cannot be a homozygote (rr). It is of course possible that he may be a heterozygote, especially in view of his breeding. If this is the case then the outstanding clinical feature, viz. the photosensitization, which first threw suspicion on this animal as a porphyrin sufferer, must be caused by something other than the usually recognised porphyrins.

As already stated this animal died either from heartwater itself or as a result of haemolysis induced by the uleron treatment for heartwater. It was therefore possible to make a complete postmortem examination and also to examine the various organs pathologically.

Post Mortem Examination.

Lesions of photosensitization are present in places along the side of the body in the thoracic region; the skin is hairless. In other places raw surfaces are present but there is no keratinization; there is tick infestation including *Amblyomma hebraeum* (bont tick); tumour splenis; enlarged liver, hydrothorax, with petechiae on the epicardium; marked gelatinous infiltration of the pulmonary septa and marked oedema of the lungs, degenerative changes of the kidneys; haemoglobinuria, enlargement of the lymphatic glands.

There is no discoloration of the teeth or the bones, consequently the lesions of Photosensitization are the only macroscopic evidence of pink tooth. the other evidence suggestive of porphyria, especially in view of the photosensitization which is present, is the fact that the bull is related to the porphyrin carrier bull 7015 referred to by Fourie (1936, 1939).

The anatomical-pathological diagnosis is typical of heartwater, which disease the animal contracted naturally. The haemoglobinuria has however nothing to do with the heartwater infection, but was caused by the uleron, which drug is a useful curative remedy for heartwater, if used during the early stages of the disease according to Neitz (1940).

In order to obtain further evidence as to whether this is a typical case of porphyria or not, a careful histological examination of representative organs was made. Details of the examination are:—

Myocardium.—Numerous haemorrhages are present. These are irregularly distributed throughout the myocardium. Sarcosporidia are frequent. No well-defined evidence of undoubted pigmentation is present.

Bronchial Lymphatic Glands.—There is a small amount of pigment appearing almost dustlike in cells (8 mm. lens × 6 ocular), but the granular nature of this pigment is clearly seen with a 4 mm. dry lens. The pigment stains brown with haem-eosin and dark brown or almost black with v. Gieson. The pigment does not take the Sudan stain, appearing merely as refractile points with this stain. In addition to this granular pigment, there are also bigger pigment masses, staining blue with Berliner Blue. There is not much of this pigment, which is regarded as haemosiderin, due to haemolysis induced by the uleron injections.

Mediastinal Lymphatic Glands.—Pigmentation as described for the bronchial glands is present, but to a much smaller extent.

Spleen.—A good deal of blood is present in the spleen. In places pigment masses staining of a yellowish brown colour, with haem-eosin are present. With Berliner Blue, a great deal of iron staining pigment is seen to be present. (Fig. 2 and Fig. 3.)

There is undoubtedly more iron containing pigment in the spleen of this animal than is the case with the bovine cases referred to by Fourie (1936). But this iron containing pigment is not due to the same aetiological factors, described for the case Petry by Borst and Königsdorffer (1929), but rather due to the haemoglobinaemia induced by the Uleron treatment for heartwater. The finely granular pigment seen in typical cases of congenital porphyrinuria was not recognised.

Lungs.—There is marked oedema. In places the alveoli contain fibrin. Some dark, almost black pigment masses of varying size are sometimes seen. This pigment appears to be situated largely in the interstitial tissues and is regarded as consisting of carbon particles, the condition being actually anthracosis. There is in addition a good deal of iron staining pigment in the lungs.

Liver.—Some pigment is present, but most of it stains for iron with Berliner Blue and is regarded as being due to the haemoglobinaemia.

Kidneys.—Haemorrhages, small in extent, are present throughout the substance of the kidneys. In some tubules a structureless fluid staining reddish with eosin is present (haemoglobin). In some glomeruli a similar substance is present. There is also a certain amount of iron staining pigment present. These changes are due to heartwater (haemorrhages) and to the effects of the Uleron treatment (haemoglobinaemia), but undoubted porphyrin pigments were not recognised.

The macroscopic and microscopic appearances of the lesions described are typical for heartwater complicated with haemoglobinaemia. There is no undoubted evidence that porphyrin pigments are present. This is further confirmed by the work of Roets (unpublished results) who was not able to find any porphyrins in the bones of this animal. Roets, however, found as much as ·7 mg. of coproporphyrin in 100 gm. of rectal faeces (post mortem). Compare this with 3·446 mg. of coproporphyrin per 100 gm. of faeces and 6·443 mg. per litre of urine in a severe porphyrin sufferer No. 7018 and 2·813 mg. coproporphyrin per 100 gm. of faeces and 4·88 mg. coproporphyrin per litre of urine in a less severe case No. 7016. [See Fourie and Roets (1939)].

There was no uroporphyrin in the urine. It is incidentally worthy of note that there was as much as ·399 gm. of haemoglobin per 100 c.c. of urine (in bladder post mortem). These amounts of coproporphyrin in the faeces and urine are considerably higher than those recorded by Fourie and Roets (1939) when quantitative coprophyrin determinations were made from samples of faeces and urine collected from this animal when alive and not suffering from an acute disease like heartwater or complications such as haemoglobinaemia. The figures obtained by them at that time are—coproporphyrin $\pm$ ·15 mg. for 100 gm. of faeces and plus minus ·055 mg. per litre of urine.

Roets further found in bull 7597: (1) *Spleen*, chloroform soluble porphyrins 50 γ per kilo, coproporhpyrin traces.

(2) *Kidney*—no porphyrins.

(3) *Liver.*—Chloroform soluble porphyrins 184 γ per kilo and coproporphyrin 131 γ per kilo.

(4) *Bile*—Chloroform soluble porphyrin 48 γ per kilo and coproporphyrin 102 γ per kilo.

It is not known how to interpret these findings. These figures undoubtedly lose much in value in view of the fact that figures for normal animals as well as for animals suffering from various diseases are not available for comparison. This is a study which has already been undertaken by one of my colleagues at Onderstepoort but sufficient data are not yet available to allow of a comparison being made. Although it is not possible to interpret the above figures at present, it was nevertheless decided to record them for the sake of future workers. In this connection one should, however, remember that already in 1892 Garrod had shown that the substance he refers to as haematoporphyrin is frequently present in minute amounts in healthy individuals and in large amounts in urines of sufferers from a great variety of diseases.

Discussion.

This interesting case, bull 7597, has a remote common ancestry with a known porphyrin carrier bull 7015. It was further known to have had lesions of photosensitization from calfhood. Its total daily porphyrin excretion is very much lower than is the case with other known bovine porphyrin sufferers, but appears to be on a somewhat higher level, than is the case with normal animals. This evidence suggests that this animal may be a case of porphyria. However, since the teeth, bones and urine of this animal are not discoloured as is the case with typical pink tooth cases in bovines it is possible that this animal may be an atypical case of porphyria.

The evidence against porphyria is, on the other hand, undoubtedly impressive. This briefly is:—There are no porphyrins in the bones. There is no uroporphyrin in the urine. there is no discoloration of the teeth or the bones; when bred to two undoubted pink tooth females, both calves born as a result of this mating are clinically normal. This genetical evidence would therefore seem to confirm the evidence furnished by (i) chemical analysis of urine, faeces and organs and (ii) macroscopic and microscopic examination of representative organs, that this animal, in spite of being in a continuous state of photosensitization, is not a porphyrin sufferer, or if it is a case of porphyria, it must be an atypical and hitherto unrecognised type of porphyrin sufferer.

Summary.

A Shorthorn bull 7597 remotely related to a porphyrin carrier (Dr) bull (7015) and showing skin lesions of photosensitization from calfhood, was bred to two cows suffering from congenital porphyrinuria (pink tooth). Both calves born out of this mating are clinically normal. The bull died from heartwater or haemoglobinuria induced by Uleron treatment for heartwater. Histologically no undoubted evidence of porphyria was recognised. Chemically no porphyrins were found in the bones and no uroporphyrin in the urine. The teeth are not discoloured. This chemical and histological evidence tends to confirm the genetical evidence that this animal is not a porphyrin sufferer, or, if it is, it must be a very unusual and atypical case of porphyria.

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Fig. 1. Bull 7597, showing acute lesions (photosensitization).

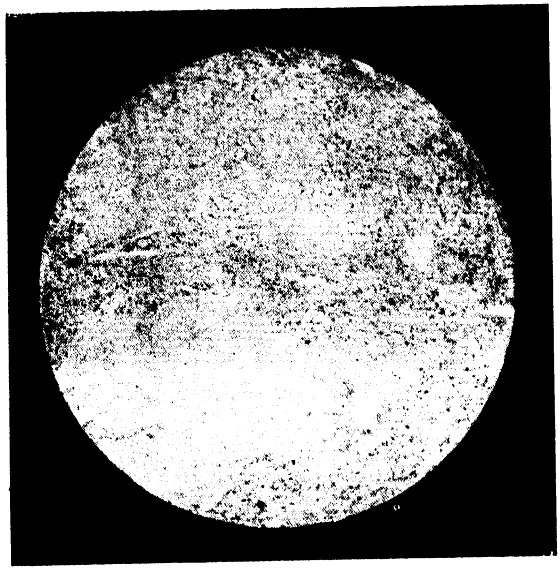

Fig. 2.—Spleen 20×, showing iron staining pigment. Berliner Blue

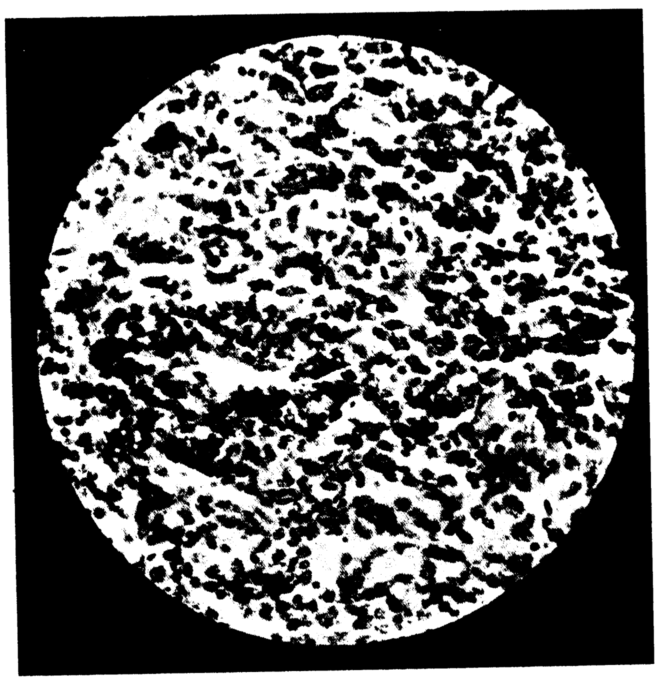

Fig. 3.—As fig. 2. ×330.

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Chemical Blood Studies. IX—The Fractional Determination of the Acetone Bodies in Blood and Urine of Sheep Suffering from Domsiekte.

By J. R. MALAN, Section of Chemical Pathology, Onderstepoort.

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I. INTRODUCTION.

DUE to the fact that acetonaemia is being encountered more frequently lately in veterinary practice and, in South Africa is manifested in Domsiekte (Pregnancy Disease) in sheep, the occasion arose to study the acetone bodies in the blood and urine of sheep suffering from Domsiekte.

Since, as far as is known, no attempt has been made as yet to separate the acetone bodies in Domsiekte into their different components, this aspect was also considered. Determinations were done on both blood and urine of the acetone, plus the acetoacetic acid fraction by the Messinger technique, and of β-hydroxybutyric acid by the same technique, after oxidation by the Shaffer method, using certain modifications as will be noted later.

II. EXISTING METHODS.

A. *Removal of Proteins, Glucose and Other Interfering Substances.*

Before the acetone bodies can be estimated in both blood and urine, the proteins in the former and the interfering substances, such as glucose, paired glucuronic acids, aldehydes, hydrogen sulphide and volatile acids, such as lactic and formic, in both have to be removed.

To achieve this, when using blood, the following procedures are adopted:—

Marriot (1913c) used a modification of the Seegen procedure of sodium acetate precipitation for removal of the proteins and subsequent treatment of the distillate with basic lead acetate and ammonium hydroxide; van Slyke and Fitz (1917) and Barnes (1937) used a solution of mercuric sulphate which removes both proteins and other interfering substances; Behre and Benedict (1926) employed the Folin-Wu method of sodium tungstate and sulphuric acid for removal of the proteins, followed by the van Slyke (1917) procedure, consisting of copper sulphate and calcium hydroxide when β-hydroxybutyric acid is also to be determined.

Kennaway (1918) used lead acetate and ammonia, as recommended by Shaffer (1908) and as used by Kennaway (1914) for urine, for the removal of both the proteins and the interfering substances; Hubbard (1921c) used colloidal iron and basic lead acetate, followed by sodium hydroxide to precipitate the lead and to remove not only proteins but also other interfering substances. It may be here mentioned that van Slyke and Fitz (1917) stated in a footnote that colloidal ferric hydroxide when added to blood gave a beautiful protein-free filtrate, but that the precipitate absorbed about one-third of the β-hydroxybutyric acid present.

Widmark (1919) describes a micro-method for estimation of acetone in blood in which the blood is distilled with one per cent. phosphoric acid and the acetone evolved passed directly into the alkaline iodine solution of the Messinger method.

For the removal of the glucose and interfering substances in urine Shaffer (1908) recommended the use of basic lead acetate and ammonium hydroxide. This procedure has also been adopted by Shaffer and Marriot (1913) and Kennaway (1914). Van Slyke (1917) employed copper sulphate and calcium hydroxide and this latter procedure was also used by Behre and Benedict (1926) and van Slyke (1929). Hubbard (1921B) combined these two procedures to use lead acetate and copper sulphate, followed by sodium hydroxide, instead of ammounia.

B. *Purification of Acetone after Removal of Interfering Substances.*

In the blood and urine, after the proteins, glucose and certain of the interfering substances have been preliminarily removed, and the preformed acetone plus that derived from the aceto-acetic acid has been distilled over, usually from a sulphuric acid medium, a further purification is essential before the actual estimation can be carried out, if the Messinger procedure is adopted.

Shaffer (1908) added sodium hydroxide and hydrogen peroxide to the first distillate to hold back the volatile acids and to oxidise the aldehydes to the corresponding acids in the final distillation. This method has been adopted by Shaffer and Marriot (1913), Marriot (1913) and others. Folin and Denis (1914), used sodium peroxide instead of hydrogen peroxide for this purpose and this latter procedure has been enlarged upon by Hubbard (1921), who accomplished further purification by redistilling first from a solution of acid plus potassium permanganate and then from a sodium peroxide solution.

C. *Final Estimation of the Acetone Bodies.*

When the preformed acetone, plus that derived from aceto-acetic acid has been distilled over as acetone, and also the β-hydroxybutyric acid oxidised to acetone (see D, determination of β-hydroxybutyric acid) and purified as necessary, the final estimation can be carried out.

The Messinger (1888) titration method has been mostly used for this estimation. This method depends on the formation of iodoform from acetone in an alkaline iodine solution, the excess iodine from the known amount added, is determined, after acidification, with a standard sodium thiosulphate solution:—

$$CH_3COCH_3 + 3I_2 + 4\ NaOH = CHI_3 + CH_3COONa + NaI + 3\ H_2O$$

Excess Iodine:—

(*a*) Alkaline medium...... $2\ NaOH + I_2 = NaI + NaIO + H_2O.$

(*b*) Acid medium......... $NaI + NaIO + 2\ HCl = 2\ NaCl + H_2O + I_2.$

(*c*) Acid medium.......... $2\ Na_2S_2O_3 + I_2 = Na_2S_4O_6 + 2\ NaI.$

Decinormal solutions of iodine and thiosulphate are usually used and from the first equation since—

$$1\text{ c.c. } \frac{N}{10}\ I_2 = 1\text{ c.c. } \frac{N}{10}\ CH_3COCH_3.$$

$$1\text{ c.c. } \frac{N}{10}\ I_2 = \frac{(58\cdot048 \times 1000)}{(6 \times 10 \times 1000)}\text{ mgm.}$$

$$= 0\cdot9675\text{ mgm. acetone.}$$

The method has been proved by Marriot (1913a) and others to be accurate even when using dilute solutions of acetone. The method has been employed by Folin (1907), Stuart-Hart (1908), Shaffer (1908), Shaffer and Marriot (1913), Marriot (1913), Hurtley (1916), Widmark (1919), Hubbard (1920 and 1921) and others.

Another method is that proposed in 1898 by Denigés, depending on the formation of a compound of acetone and mercuric sulphate. The compound, which was crystalline could be weighed or the mercury content could be estimated by titration with silver nitrate and potassium cyanide. This method was adopted by van Slyke (1917) and van Slyke and Fitz (1917) using the titration method of Personne (1863), the precipitate being dissolved in hydrochloric acid, excess potassium iodide solution added, and the excess iodide estimated by titration with mercuric chloride.

The compound formed between the acetone and mercuric sulphate may be one of the following:—

$3\ HgSO_4\cdot 5HgO\cdot 2(CH_3)_2CO$ or

$2\ HgSO_4\cdot 3HgO(CH_3)_2CO,$

both of which contain about 77 per cent. mercury.

The reactions involved in the titrations are:—

$HgSO_4$ (or $HgCl_2$) $+ 2\ KI = K_2SO_4$ (or $2\ KCl$) $+ HgI_2$ (insoluble)
$HgI_2 + 2KI$ (in excess) $= K_2HgI_4$ (soluble)

Excess KI: $HgCl_2 + 4KI = K_2HgI_4 + 2KCl.$

Barnes (1937), after dissolving the acetone-mercury compound in acid, distilled the acetone with heat into an alkaline iodine solution and titrated the excess with thiosulphate (Messinger).

A colorimetric method based on the reaction between acetone and salicylic aldehyde with the formation of a coloured product, dihydroxydibenzene acetone was introduced by Engfeldt (1915), based on the qualitative method of Frommer (1905):—

$$2C_6H_4(OH)CHO + CH_3COCH_3 = [C_6H_4(OH)CHCH]_2CO + 2H_2O.$$

Csonka (1916) did not find the method applicable to small quantities of acetone, but Behre and Benedict (1926), however, overcame this drawback by substituting an alkaline solution for the alcoholic solution of the aldehyde employed by Csonka. The method has also been used by Urbach (1931) and Neuweiler (1933).

A further method, by Scott-Wilson (1911), is based on the formation of a double compound of acetone and basic mercuric cyanide in an alkaline medium, with subsequent titration of the mercury present in the precipitate with sulphocyanate, after decomposition of the precipitate with nitric acid and permanganate. Scott-Wilson gives the reaction for the precipitation as:—

$$CH_3COCH_3 + 2\ Hg(CN)_2 + 3\ HgO = HgCOC_2(HgCN)_4 + 3\ H_2O$$

This method has been adopted by Marriot (1913b) for the determination of very small amounts of acetone by measuring the precipitate with a nephelometer, and by Folin and Denis (1914) who adopted it for using in a Duboscq colorimeter.

D. *The Determination of β-Hydroxybutyric Acid.*

The history as regards the discovery and the subsequent investigation into the properties and determination of β-hydroxybutyric acid has been discussed and enlarged upon by Shaffer (1908), Hurtley (1916) and Hubbard (1921a).

The next method was based upon the optical activity of the laevorotatry greater than that theoretically needed to neutralize the inorganic acids found in the urine, as β-hydroxybutyric acid, was necessarily inaccurate as it merely expressed the amount of organic acidity (Stadelmann, 1883).

The next method was based upon the optical activity of the laevorotatory acid or its salts after fermentation of the urine (Kulz, 1884). The results were, however, almost of no value and extraction of the acid by ether was first used by Wolpe (1886), and improved by Magnus Levy (1901), Bergell, and Black (1908). Black dehydrated the evaporated urine with plaster of Paris and employed a continuous ether extraction apparatus, finally determining the strength in the polariscope. Hurtley (1916) employing the method discarded the addition of ammonium sulphate as recommended by Magnus Levy (1901) and Geelmuyden (1906), as it was of no real advantage.

A further method was that of Darmstädter (1903), who evaporated the alkalinised (Na_2CO_3) urine and then distilled at constant concentration of 50 per cent. sulphuric acid to convert the β-hydroxybutyric acid into α-crotonic acid, which was extracted with ether and after removal of volatile acids was estimated by titration.

The inadequacy of these methods led Shaffer (1908), to propose the oxidation of the β-hydroxybutric acid with potassium dichromate and sulphuric acid to acetone and carbon dioxide, with the usual Messinger estimation of the former.

This method appears to be the most reliable and has certainly been used extensively. The oxidation is, however, not quantitative, and although it has been improved and enlarged upon by Shaffer and Marriot (1913), Marriot (1913), Folin and Denis (1914), van Slyke (1917) and Hubbard (1921) and improvements brought on as regards time employed and its adaptability, it is apparent that at its best a yield of 90 per cent. can be considered as the optimum.

III. Experimental.

A. *Is Lead Acetate Treatment Compulsory?*

Since in Domsiekte we find a condition of hypoglycaemia, in direct contrast to that seen in diabetes, coupled with hyperacetonaemia in both, and since we failed to find any glucose in the urine in Domsiekte, the question arose whether it was necessary to remove preliminarily the remaining interfering substances.

Shaffer (1908) in formulating his method for the oxidation of β-hydroxybutyric acid with chromic acid noted that the chief danger in not applying the lead acetate treatment lay in the conjugated glucuronic acids, which on oxidation with the chromic acid also gave rise to acetone

Spaeth-Kaiser (1936) mentions that the paired glucuronic acids are formed chiefly, both as a result of incomplete sugar oxidation and in marked respiratory disturbances as are manifested in diabetes mellitus. Since these primary causes are not seen in Domsiekte, it appeared natural to assume that the excretion of glucuronic acids in Domsiekte was at a minimum.

The acetone bodies in Domsiekte urine were thus determined separately on the same sample, treating one aliquot with basic lead acetate and ammonia and omittng it on a second portion. The acetone plus acetoacetic acid was determined first after acidification with sulphuric acid and the β-hydroxybutyric acid then oxidised with chromic acid. The distillates in all cases wery subsequently redistilled after the addition of caustic soda and hydrogen peroxide, and Messinger procedure carried out on the final distillates. Results were as follows; data expressed as c.c. decinormal iodine on the aliquot urine used.

Urine Sample.	Aliquot.	Treatment.	Acetone plus Acetoacetic.	β-Hydroxybutyric Acid.
A........................	10 c.c.	Without lead..........	15·70	12·85
		With lead.............	15·69	12·88
B........................	20 c.c.	Without lead..........	13·25	6·50
		With lead.............	13·05	6·63
C........................	100 c.c.	Without lead..........	4·45	—
		With lead.............	4·44	—

The Kennaway (1918) procedure of basic lead acetate and ammonia as applied to blood was checked against the Folin-Wu system of sodium tungstate and sulphuric acid for the removal of the proteins with subsequent redistillation of all the distillates with caustic soda and hydrogen peroxide. Results were as follows, data expressed as c.c. decinormal iodine per 25 c.c. blood, the aliquot used:

	Treatment.	Acetone plus Acetoacetic.	β-Hydroxy-butyric Acid.
Blood A	Without lead	4·90	5·90
	With lead	4·70	5·20
Blood B	Without lead	6·70	8·95
	With lead	6·60	8·85
Blood C	Without lead	11·90	13·90
	With lead	11·80	14·00

The values obtained were thus quite accurate enough to warrant the omission of the lead acetate treatment in determining the acetone bodies in the blood and urine of sheep suffering from Domsiekte.

In this connection it may be noted that Folin and Denis (1914), using the turbidity method also eliminated the "rather tedious" preliminary treatment with basic lead acetate and ammonia. They found that all the oxidation products with chromic acid which pass into the final distillate and which react with the Scott-Wilson acetone reagent, are destroyed or removed by the second distillation with the alkaline peroxide mixture. They also state that in urines containing no sugar, as in children, and in that obtained from fasting persons the determination of β-hydroxybutyric acid requires no second distillation, but that the addition of sodium peroxide to the first distillate suffices to remove all the disturbing effects of the normal urinary constituents.

B. *Methods Used.*

The methods used may be summarized as follows:

1. *Determination of Preformed Acetone plus Acetone from Acetoacetic Acid.*

The blood proteins were removed by the Folin-Wu system of tungstic acid precipitation, using the strengths as modified by Graf (1933), namely 11 per cent. sodium tungstate ($Na_2WO_4 2H_2O$) and 0·725 normal sulphuric acid. The distilled water, tungstate oxalated blood and acid (in sequence mentioned) were used in proportions of 7 : 1 : 1 : 1 and after thorough mixing were filtered through Schleicher and Schüll's folded filter paper No. 588, no difficulty being encountered in obtaining clear filtrates.

From 100-250 c.c. filtrate for blood and from 5-100 c.c. of urine were used for an analysis. The Rothera's Reaction, consisting of a mixture of powered ammonium sulphate and a one per cent. sodium nitroprusside which is added to a small aliquot of urine, followed by the addition of ammonium hydroxide—a permanganate colour indicating a positive case— was employed to give an indication of the severity of acetonuria, which indirectly gave also an indication of the degree of acetonaemia.

The aliquot used was diluted to about 300 c.c. with water, 10 c.c. 1:1 sulphuric acid added and distillation carried out for at least 40 minutes, but avoiding a longer period to prevent splitting off of acetone from the β-hydroxybutyric acid (van Slyke 1917). The receiver was previously charged with water, and delivery tube allowed to dip below the surface. With a medium flame from 200 to 250 c.c. distillate was collected, water being added from a dropping funnel to distilling flask to keep the volume up to 150 c.c.

The distillate which contained the preformed acetone, plus that derived from the acetoacetic acid,

$$CH_3COCH_2COOH = CH_3COCH_3 + CO_2 \text{ or its ester}$$

$$CH_3CO\ CH_2\ COOC_2H_5 + H_2O = CH_3COCH_3 + C_2H_5\,OH + CO_2$$

was redistilled after the addition of 5 c.c. of about 30 per cent. caustic soda solution and a few c.c. of a 6 per cent. hydrogen peroxide, distillation being continued for about 30 minutes with same precautions as given above as regards the receiver, to give approximately 150 c.c. distillate.

The acetone was estimated on this final distillate by the Messinger technique as follows: To the distillate was added 5 c.c. 40 per cent. sodium hydroxide solution, and after mixing, 20 c.c. standard iodine solution accompanied with further mixing. After standing for 15 minutes with occassional shaking, 10 c.c. 25 per cent. hydrochloric acid was added, and the excess iodine thus liberated was titrated with a standard thiosulphate solution, using starch as indicator.

The difference in c.c. between the volume of iodine solution added and the thiosulphate solution used (standards being both exactly decinormal) multiplied by 0·9675 gives the acetone content in mgm.

2. *Determination of β-Hydroxybutyric Acid.*

The β-hydroxybutyric acid was estimated on the same sample as that used for estimation of acetone as described above.

To the residue in the distilling flask was added 25 c.c. of a solution containing 2 per cent. potassium dichromate and 35 per cent. sulphuric acid as recommended by Folin and Denis (1914). The volume of flask was made up to about 300 c.c. and distillation carried out for about 45 minutes, with the same precautions as given above for acetone. The β-hydroxybutyric acid was hereby oxidised to acetone.

$$CH_3CHOHCH_2COOH + O = CH_3COCH_3 + CO_2 + H_2O.$$

The 25 c.c. dichromate reagent proved to be ample for all blood filtrates, but in the urine samples dilute potassium dichromate had to be added occasionaly through the dropping funnel when solution tended to assume a too predominantly green colour. The volume was not allowed to sink below 150 c.c.

The distillate thus obtained was redistilled and the estimation carried out as for acetone determination.

3. *Determination of Total Acetone Bodies.*

In a few cases in both blood and urine, the time available did not allow a separation of the acetone bodies into the fractions, and a "total acetone" determination had to be done.

As in the above determinations 10 c.c. 1:1 sulphuric acid was added to the aliquot of the blood filtrate or to the urine sample and the contents of flask boiled for about 10 minutes, with the receiver attached, before the chromic acid was added. This was done to enable the acetoacetic acid to resolve into acetone and carbon dioxide as otherwise low results were obtained. Distillation was hereafter carried on for a further 45 minutes and after redistillation as above, the final estimate was carried out, using Messinger technique.

For all determinations apparatus with glass ground joints were used throughout.

IV. Discussion and Results.

Some of the values obtained by this method are tabulated below. Two sheep, in the last month of pregnancy, are considered, both as normal and pathological for both blood and urine. All values are expressed in terms of acetone as mgm., in blood, per 100 c.c., and in urine, either as per 100 c.c., or as the total output of urine for the 24 hours period over which collection was carried out. The urine was always collected for the 24 hours period following immediately after the time of bleeding.

Abbreviations used:

Acet. for acetone,

A.A. for acetoacetic acid,

H.B.A. for β-hydroxybutyric acid.

Particulars regarding sheep:— Sheep A was placed on veld hay diet on 12 April and she aborted on 28 April. For sheep B these dates are 8 March and 25 March respectively.

No.	Date.	Blood.			Urine.					
						Acet. + A.A.		H.B.A.		Total Acetone Bodies per 24 hours.
		Acet. + A.A.	H.B.A.	Total Acetone Bodies.	c.c. Urine.	Per 100 c.c.	Per 24 hour.	Per 100 c.c.	Per 24 hour.	
	1940									
A.	12/4	1·45	2·90	4·4	910	—	—	—	—	39·6
	15/4	3·09	4·25	7·4	295	47·4	139·8	8·1	23·9	163·7
	18/4	6·58	6·96	13·6	640	140·2	897·3	10·6	67·8	965·1
	22/4	11·99	10·44	22·4	560	243·7	1,364·7	15·5	86·8	1,451·5
	25/4	7·35	16·25	23·7	450	301·7	1,357·7	68·7	309·2	1,666·9
	29/4	1·93	3·87	5·8	—	—	—	—	—	—
B.	8/3	0·4	1·3	1·7	445	2·6	11·6	3·7	16·5	28·1
	18/3	14·8	13·2	28·0	665	318·1	2,115·4	31·9	212·1	2,327·5
	20/3	12·3	11·9	24·5	370	365·5	1,352·4	51·3	189·8	1,542·2
	23/3	—	—	37·9	610	318·1	1,940·4	39·7	242·2	2,182·6
	26/3	—	—	4·3	66	—	0·7	—	1·0	1·7

The rest of the data obtained from sheep, both normal and suffering from Domsiekte, by this method are published in the article Domsiekte, or Pregnancy Disease in Sheep, II, by Groenewald, Graf, Bekker, Malan and Clarke (1941).

V. Summary.

Methods for the determination of the acetone bodies in both blood and urine in sheep suffering from Domsiekte are given. The preliminary treatment with basic lead acetate and ammonia, has been found to be of no advantage in this disease and has been omitted.

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The Recovery of Potassium Carbonate from Wool Scouring Liquor and the Conversion of the Recovered Potassium Carbonate to Commercial Potassium Sulphate, using Naturally Occurring Gypsum as Sulphate Source.

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In studying ways and means of converting, on a commercial basis, an alkali carbonate such as potassium carbonate to the corresponding alkali sulphate, one should bear in mind that the success of the conversion should be studied from the viewpoint of the efficiency of the cation exchange and not in terms of total potassium. The presence of potassium should be regarded as being merely incidental. Furthermore it has been found in the present study, contrary to analytical figures for wool suint from other parts of the world, that South African scouring liquors contain sulphate. In all probability the sulphate is contained in the scouring liquor as potassium sulphate. If potassium sulphate is not normally excreted through the skin of the sheep its presence in wool suint will naturally range from mere traces to considerable quantities.

The presence of potassium sulphate in wool suint may feasibly be ascribed to (*a*) a sulphate containing ground and a sulphate containing burned veld ash contamination of the wool; (*b*) the dipping of sheep in sulphate-carrying waters. Under these conditions the reaction:—

$$MSO_4 + K_2CO_3 = MCO_3 + K_2SO_4 \quad (M = Ca, Mg)$$

will gradually proceed in the wool suint. The presence of potassium chloride in wool suint has been known for a long time.

On account of the reasons stated above the problem was tackled by establishing in the first instance the initial active carbonate and sulphate content of the ash obtained by the destructive combustion of wool scouring liquor concentrate.

Analysis of ash.

34·37	per cent. active carbonate.
=79·16	per cent. in terms of potassium carbonate.
1·795	per cent. active sulphate.
= 3·258	per cent. in terms of potassium sulphate.
3·505	per cent. active chloride.
= 7·370	in terms of potassium chloride.

Analysis of gypsum.

45·81 per cent. available SO_4 (conversion by Na_2CO_3).
44·34 per cent. available SO_4 (conversion by K_2CO_3).
45·58 per cent. available SO_4 (conversion by $(NH_4)_2CO_3$).

Average = 81·14 per cent. $CaSO_4 \cdot 2H_2O$.

Acid insoluble residue = 16·05 per cent. (sand).

Experiment A.

The conversion efficiency of carbonate to sulphate was studied in solutions obtained by the leaching of concentrated scouring liquor ash. The solutions adopted in this study contained an active carbonate concentration of 0·143 per cent. to 8·56 per cent. in terms of carbonate. By working from the equation:—

$$M_2CO_3 + CaSO_4 \cdot 2H_2O = M_2SO_4 + CaCO_3 + 2H_2O.$$

the stochiometric quantity of powdered gypsum was added to each specific solution, the mixtures gently boiled for 30 minutes. (Tests were run in 250 ml. volumes.) After the solutions had been allowed to cool it was filtered, washed and the filtrate made up to standard volume. The sulphate, residual calcium and carbonate were determined in aliquots from the unit volume. The sulphate was determined gravimetrically, the calcium volumetrically by permanganate titration and the residual free carbonate by an accurate two-stage titration. In this way the efficiency of the cation exchange was checked from three sides.

The results are tabulated in Table 1.

Table 1.

A.	B.	C.	D.	E.	F.	G.	H.	I.	J.
1·0347	1·257	0·0452	8·30	2·30	1·3292	1·284	0·330	0·356	92·7
3·0235	3·671	0·1320	29·90	2·90	3·8220	3·690	0·949	1·040	91·2
6·0435	7·340	0·2639	68·80	1·75	7·5179	7·254	1·865	2·077	89·8
9·0051	10·930	0·3931	98·90	3·40	11·2530	10·860	2·791	3·094	90·2
11·9824	14·550	0·5232	145·5	2·70	14·8132	14·290	3·673	4·118	89·2
25·8500	31·390	1·1291	376·4	2·10	30·5390	29·411	7·560	8·710	86·8

A = Weight of ash per 250 ml.
B = 81·14% Gypsum equivalent of A.
C = $BaSO_4$ equivalent of the initial sulphate content.
D = Residual free carbonate in ml. O.I.N.
E = Residual free calcium in ml. O.I.N.
F = Total weight of $BaSO_4$.
G = $BaSO_4$ equivalent of potassium sulphate formed by cation exchange.
H = CO_3 equivalent of potassium sulphate formed by cation exchange.
I = Available CO_3.
J = Percentage conversion of carbonate to sulphate.

The conversion percentages arrived at in Table 1 are not absolute, as the $BaSO_4$ equivalent of the residual calcium in column E was not allowed for. However, this amount is almost entirely negligible.

Experiment B.

The experiment carried out in A was repeated on a further 250 ml. aliquot of the original leach. Gypsum was now added in an excess of 100 per cent. over and above the stochiometric equivalent.

This large excess of gypsum was adopted in order to study the solubility of the excess gypsum in solutions with a progressive increase in potassium sulphate concentration. The results are tabulated in Table 2.

TABLE 2.

A.	B.	C.	D.	E.	F.	G.	H.	I.	J.	K.
1·0347	2·514	3·20	0·1531	1·8342	0·447	1·342	0·345	0·356	0·0452	96·92
3·0235	7·342	1·80	0·2183	4·7930	0·6368	4·023	1·034	1·040	0·1320	99·42
6·0435	14·680	0·25	0·3119	9·2372	0·9101	8·063	2·084	2·077	0·2639	100·3
9·0051	21·860	5·5	0·3839	13·4830	1·120	11·970	3·076	3·094	0·3931	99·19
11·982	29·120	0·34	0·4397	16·9732	1·283	16·167	4·157	4·118	0·5232	101·1
25·850	62·780	37·5	0·4793	35·947	1·398	33·420	8·592	8·710	1·1291	98·64

A = Weight of ash per 250 ml. volumes.
B = 81·14% Gypsum equivalent + 100% excess.
C = Residual free carbonate in ml. O.I.N.
D = Residual calcium in grams per 1,000 ml.
E = Total weight of $BaSO_4$.
F = $BaSO_4$ equivalent of the residual calcium.
G = $BaSO_4$ equivalent of the potassium sulphate formed by cation exchange.
H = CO_3 equivalent of the potassium sulphate formed by cation exchange.
I = Available CO_3.
J = $BaSO_4$ equivalent of the initial sulphate content.
K = Percentage conversion of carbonate to sulphate.

Experiment C.

Scouring Liquor Concentrate.

Scouring liquor was concentrated, evaporating 4 volumes of water per every initial 5 volumes of liquor. The liquor contains at this stage 2·649 grams of available carbonate per 100 grams of solution and a sulphate content of 0·208 per cent. in terms of sulphate.

The liquor was mixed with (*a*) the equivalent amount of powdered gypsum, (*b*) gypsum in 10 per cent. excess of the theoretical amount, and (*c*) gypsum in a 100 per cent. excess.

The mixtures were heated past the frothing stage, gradually combusted and finally ashed at dull red heat allowing sufficient air contact.

The results for the conversion of carbonate to sulphate are tabulated in Table 3.

TABLE 3.

A.	B.	C.	D.	E.	F.	G.	H.	I.	J.	K.
23·4375	2·196	2·1515	·0464	40·9	·1185	0·124	1·909	·4909	·6209	79·1
20·707	2·134	2·5000	·1120	5·1	·1047	0·327	2·067	·5325	·5488	97·5
22·581	4·230	3·3020	·3020	1·2	·1142	0·881	2·308	·5933	·5981	99·2

A = Weight of liquor.
B = Weight of gypsum.
C = Total weight $BaSO_4$.
D = Residual Ca in grams per 1,000 ml.
E = Residual CO_3 in ml. O.I.N.
F = $BaSO_4$ equivalent of sulphate initially present.
G = $BaSO_4$ equivalent of the residual calcium.
H = $BaSO_4$ equivalent of potassium sulphate formed by cation exchange.
I = CO_3 equivalent of potassium sulphate formed by cation exchange.
J = Available CO_3.
K = Percentage conversion of carbonate to sulphate.

EXPERIMENT D.

Centrifuged (Middle Portion) of the Concentrated Scouring Liquor.

The concentrated scouring liquor as used in Experiment C was centrifuged by Mr. Rossouw at approximately 4,000 r.p.m. for 60 minutes. The middle layer was used in this experiment for the recovery of potassium. This layer contained an available carbonate concentration of 2·826 grams CO_3 per 100 grams liquor and a sulphate content of 0·221 per cent. on a weight basis.

This middle layer should naturally be higher in both carbonate and sulphate content than the initial total fraction (see Experiment C) as the middle layer lost a considerable amount of fat and semi-colloidal material. The experiment as described under C was carried out on this middle fraction. The results are tabulated in Table 4.

TABLE 4.

A.	B.	C.	D.	E.	F.	G.	H.	I.	J.	K.
19·8042	1·979	1·9790	·0314	31·7	0·1064	0·0916	1·781	0·4580	·5595	81·84
18·9518	2·083	2·4235	·0929	2·2	0·1018	0·2707	2·051	0·5274	·5356	98·5

A = Weight of liquor.
B = Weight of gypsum.
C = Total weight of $BaSO_4$.
D = Residual calcium in grams per 1,000 ml.
E = Residual carbonate in ml. O.I.N.
F = $BaSO_4$ equivalent of sulphate initially present.
G = $BaSO_4$ equivalent of the residual calcium.
H = $BaSo_4$ equivalent K_2SO_4 formed by cation exchange.
I = Available CO_3.
J = Available CO_3.
K = Percentage conversion of carbonate to sulphate.

Experiment E.

Cold Scouring.

The scouring of wool with cold water was carried out by Mr. Rossouw. The scouring liquor showed an available carbonate content of 0·8056 grams CO_3 per 100 grams liquor and a sulphate content of 0·0554 grams SO_4 per 100 grams liquor.

The liquor was treated with (*a*) the stochiometric equivalent of gypsum, (*b*) the equivalent of gypsum + 10 per cent. excess and (*c*) the equivalent of gypsum + 100 per cent. excess.

The mixtures were carefully heated past the frothing stage, combusted and finally ashed at dull red heat. The results for the conversion of carbonate to sulphate are tabulated in Table 5.

Table 5.

A.	B.	C.	D.	E.	F.	G.	H.	I.	J.	K.
500	15·67	12·6708	0·028	310·5	0·6491	0·0817	11·9400	3·078	4·028	76·4
500	17·237	16·1790	0·240	68·9	0·6491	0·7001	14·8298	3·813	4·028	94·65
500	31·34	17·0938	0·465	49·7	0·6491	1·355	15·1897	3·879	4·028	96·3

A = Weight liquor.
B = Weight gypsum.
C = Total weight $BaSO_4$.
D = Residual Ca in grams per 1,000 ml.
E = Residual CO_3 in ml. O.I.N.
F = $BaSO_4$ equivalent of initial sulphate.
G = $BaSO_4$ equivalent of residual calcium.
H = $BaSO_4$ equivalent of K_2SO_4 formed by cation exchange.
I = CO_3 equivalent of K_2SO_4 formed by cation exchange.
J = Available CO_3.
K = Percentage conversion of carbonate to sulphate.

Discussion.

From the analytical data obtained in the various experiments it is evident that the quantitative conversion of patassium carbonate *ex* wool scouring liquor to potassium sulphate may readily be brought about on a commercial scale by a simple cation exchange reaction using natural gypsum as a source of available sulphate.

Low conversion percentages are encountered in cases where the gypsum is added in quantities just sufficient to allow a stochiometric reaction. This may be accounted for by the possible development of a time reaction in dilute aqueous solutions or in the case of the concentrated masses it may be due to an insufficient intimate contact between the carbonate and the particles of gypsum. As soon as gypsum is added in excess the carbonate to sulphate conversion becomes quantitative. In some cases a large excess (100 per cent.) of gypsum was employed in order to study the solubility of the free gypsum in varying concentrations of potassium sulphate.

The values given for the residual calcium (Table 2, column D) is not a true reflection of the solubility of gypsum in solutions of potassium sulphate as tests were run in 250 ml. volumes which were filtered and made up to

500 ml. with cold water. Thus the figures contained in Table 2, column D is a reflection partially of the true solubility of gypsum in potassium sulphate and partially of the leaching effect of the added cold water. Actually the solubility of gypsum is decreased by potassium sulphate. The cation exchange carbonate-sulphate takes place in—

(*a*) aqueous solutions of wool scouring ash;

(*b*) concentrated scouring liquors with all the fat present or partially removed;

(*c*) scouring liquor obtained from the cold steeping of wool;

(*d*) an intimate mixture of wool scouring liquor ash and gypsum, if the mixture is heated to dull red heat.

Naturally in practice the approach to the problem of the recovery of potassium as potassium sulphate *ex* wool suint would be from the most economic angle. In this connection a few points should be carefully considered. If gypsum is added to—

(*a*) natural scouring liquor;

(*b*) concentrated scouring liquor with or without the partial removal of fat;

(*c*) mixed with the correct weight of ash, and the process of recovery culminates in ashing, great care should be exercised not to exceed the transition temperature of calcium carbonate. If the transition temperature of calcium carbonate is exceeded it is readily conceivable that the final solution containing the potassium sulphate may be contaminated with free potassium hydroxide.

If in practice a cycle process is applied, i.e., allowing an excess of carbonate with the view of using the carbonate-containing mother liquor to facilitate the scouring of wool then the presence of free potassium hydroxide would be disastrous. The presence of free hydroxyl ions in the final solution is indicated if in the two-stage acid titration the cold and hot titrations do not correspond.

It would appear that the most efficient procedure for the conversion of potassium carbonate from scouring liquor to potassium sulphate would briefly be as follows—

(*a*) a triple effect evaporation of the scouring liquor with the removal of approximately four volumes of water per every five volumes of initial liquor;

(*b*) the removal of fat by mechanical means;

(*c*) further evaporation of the middle centrifuge layer, combustion and ashing with sufficient air contact. The ashing temperature should not exceed the volatilization temperature of potassium chloride;

(*d*) the ash is leached with water, controlling the ash-water system, such that the gypsum conversion of the potassium carbonate results in a solution containing potassium sulphate in excess of the cold saturation point of potassium sulphate, i.e., crystallization would be spontaneous. The residual carbon in the ash together with the sand residue of the gypsum serve as an excellent filter aid.

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Some Aspects of Solar Radiation in its Relation to Cattle in South Africa and Europe.

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THE pioneer work of Dorno has brought to the frontline the necessity of close combination of two branches of science, namely biology and climatology (Dorno, 1911 and later publications). Dorno also was the first to stress the importance of solar radiation with regard to biological research. He included measurements of the intensity and quality of the solar radiation in the collection of climatological data, particularly for the study of the influence of solar radiation on human beings. Later on botanists realized the importance of solar radiation and extended their studies to this subject. With regard to animals, however, very little attention has been paid to solar radiation and an attempt is made in this article to apply data collected by the South African Solar Radiation Survey to cattle living in the open. For a comparison European conditions are also discussed.

The direct effect of solar radiation is twofold: firstly there is the chemical effect of the actinic rays, and secondly the heating effect. The latter only will be discussed in this article.

In order to determine the heating effect on the body surface of cattle, it is essential to know two things: (*a*) the amount of radiation impinging, and (*b*) the physical changes which this radiation will undergo when it strikes the animal. Accordingly this article will be divided into two parts:—

Part I.—The solar radiation and the factors influencing its intensity and total amount in various places.

Part II.—The amount of radiation absorbed on the body surface of cattle in South Africa and Europe.

In Part I the various factors influencing the amount of radiation obtained at various places will be discussed under the following headings:—

1. The altitude of the sun in South Africa and Europe.
2. The midday intensities of the direct solar radiation.
3. The length of the days.
4. The number of hours with bright sunshine.
5. The total amount of sun and sky radiation impinging during various months in South Africa and Europe.

In addition air temperatures will briefly be discussed.

In Part II the absorption of radiation on the body surface of cattle irradiated under South African and European conditions will be discussed under the following headings:—

1. The amount of direct solar radiation.
2. The amount of scattered radiation from the sky.
3. The amount of radiation reflected from the ground.
4. The area of the body surface which is influenced by the various kinds of radiation mentioned under No. 1-3.
6. The total amount of radiation which is absorbed on the body surface of cattle under South African and European conditions.
7. The reduction of the amount of solar radiation by natural and artificial shade.

PART I.

The Solar Radiation and the Factors Influencing its Intensity and Total Amount in Various Places.

During the period July, 1937, until June, 1938, a Solar Radiation Survey was carried out in the course of which the intensity of sun and sky radiation was recorded at six places in the Union of South Africa. (Riemerschmid, 1940). These observations were again continued in January, 1940, first at two and subsequently at four stations (Riemerschmid, 1940-41).

The geographical positions of the stations under consideration are mentioned in the appended table.

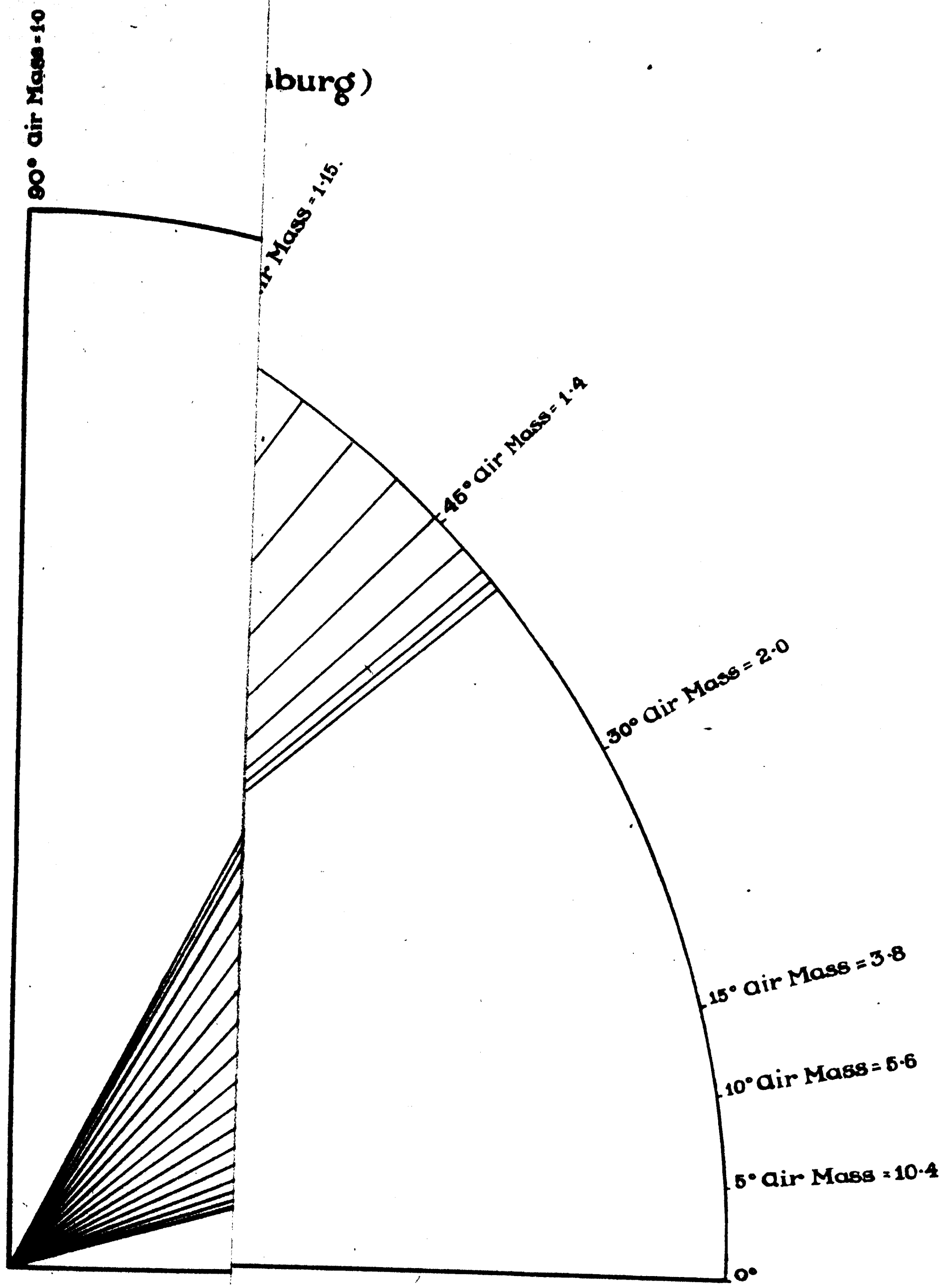
	Longitude.	*Latitude.*	*Altitude.*
1. *Inland*—			
(*a*) Johannesburg	28° 04′ E.	26° 12′ S.	5,800 ft.
(*b*) Bloemfontein	26° 13′ E.	29° 06′ S.	4,500 ft.
(*c*) Onderstepoort	28° 11′ E.	25° 38′ S.	4,000 ft.
(*d*) Armoedsvlakte	24° 39′ E.	26° 56′ S.	4,080 ft.
2. *Southern Cape*—			
(*a*) Cape Town	18° 28′ E.	33° 56′ S.	40 ft.
(*b*) Stellenbosch	18° 59′ E.	33° 50′ S.	464 ft.
(*c*) Port Elizabeth	25° 37′ E.	33° 37′ S.	250 ft.
3. *Durban*	31° 03′ E.	29° 52′ S.	20 ft.
4. *Messina*	30° 03′ E.	22° 20′ S.	1,800 ft.

The following European stations were selected for comparison with the South African stations:—

	Longitude.	*Latitude.*	*Altitude.*
1. Tunbridge Wells, Sussex	0° 16′ E.	51° 08′ N.	351 ft.
2. Aberdeen, Aberdeen	2° 06′ W.	57° 10′ N.	79 ft.
3. Kew Observatory, Richmond, Surrey	0° 19′ W.	51° 28′ N.	47 ft.
4. Davos, Switzerland	9° 49′ E.	46° 48′ N.	5,250 ft.
5. Bad Nauheim, Germany	8° 45′ E.	50° 22′ N.	492 ft.
6. Potsdam, Germany	13° 04′ E.	52° 23′ N.	323 ft.

1. *The altitude of the sun in South Africa and Europe.*

When considering the influence of radiation on any surface it must be realized that the angle of incidence influences the effect on the irradiated surface. Applying this to solar radiation as obtained in various parts of the globe, the altitude of the sun plays an important rôle.



...burg)
90° air Mass = 1·0
...ir Mass = 1·15.
45° air Mass = 1·4
30° air Mass = 2·0
15° air Mass = 3·8
10° air Mass = 5·6
5° air Mass = 10·4
0°

A demonstration of the altitude of the sun at various places seems rather difficult because it alters continuously. Each day, however, the sun reaches its maximum height (culmination) at noon solar time and the angle of culmination gives some indication of the sun heights prevailing during a large part of any day. In Fig. 1 an attempt is made to represent the culmination points of the sun graphically. In the figure each line radiating from the origin of the graph represents 20 days and the scale on the arc gives the mean altitude of culmination during these twenty days. In Sussex, for instance, the mid-winter culmination is 15°, whilst at the inland stations in South Africa the mid-winter culmination is 39°. The respective figures for mid-summer are 62° for Sussex and 87° for South Africa.

Conclusion.—The sector from which the solar rays are striking on a horizontal surface during the hours round about midday is much closer to the horizon in Europe than in South Africa. Consequently the layer of air (at equal altitudes above sea-level) between the sun and the observation station is thicker in Europe than in South Africa.

2. *Midday intensities of the direct solar radiation in South Africa and Europe.*

There are two recognised ways of measuring the intensity of the sun's energy reaching the surface of the earth. One method is to measure the solar radiation alone with an instrument which is shielded against the sky radiation. In this case the measuring surface of the instrument is, for each observation, adjusted perpendicularly to the solar beam. The other method is the measurement of the total amount of sun *and* sky radiation incident on a horizontal surface.

The midday intensities were obtained by applying the first method, i.e. by measuring the intensity at a right angle to the solar beam. Data so obtained are strictly comparative because the angle of incidence on the receiving surface is always the same. Differences in the readings are therefore due to the atmospheric conditions and their reducing effect only. The figures are significant for the transmission of the atmosphere; for biological considerations, however, sky and reflected radiation have to be included.

TABLE 1.—*Midday intensities of the direct solar radiation at various places in the Union and in Europe.*

	Durban.	Kew.		Davos.	Johannesburg.	
January	1·42	1·23	July	1·46	1·58	January.
February	1·39	1·22	August	1·46	1·56	February.
March	1·36	1·18	September	1·46	1·52	March.
April	1·32	1·09	October	1·45	1·47	April.
May	1·29	0·95	November	1·41	1·41	May.
June	1·25	0·86	December	1·36	1·38	June.
July	1·27	0·88	January	1·39	1·39	July.
August	1·32	0·99	February	1·48	1·43	August.
September	1·36	1·32	March	1·52	1·50	September.
October	1·39	1·21	April	1·51	1·55	October.
November	1·41	1·23	May	1·47	1·59	November.
December	1·43	1·23	June	1·46	1·59	December.
MEAN	1·35	1·10		1·44	1·50	MEAN.

Table 1 gives the midday intensities in gram calories per square centimetre per minute for Johannesburg (Riemerschmid, 1940) (5,800 feet above sea-level) and Davos (Lindholm, 1929) (5,250 ft.) and for Durban (Riemerschmid, 1940) (sea-level) and Kew, Surrey (Observatories Year Book, 1911-21) (47 ft.). The data given in the table were obtained from readings taken on clear days within half an hour of midday. From these data graphs showing the mean annual variation of the midday intensities were drawn up and the values for the middle of each month were read from this graph. The data are given for the corresponding months of the Southern and Northern hemisphere, namely January as compared with July (mid-summer of both hemispheres), February and August, etc.

At *low altitudes* above sea-level, namely at Durban and Kew, the comparison shows a considerable difference between the midday intensities. This difference is greatest in mid-winter, when Kew shows intensities which are up to 31 per cent. smaller than those measured at Durban. In summer the difference is still considerable; the midday intensities at Durban are approximately 13 per cent. higher than at Kew.

At *high altitudes* above sea-level (Johannesburg and Davos) the comparison shows a very small difference, amounting to 8 per cent. at the utmost, but being only 4 per cent. on an average.

Conclusion: The fact that the difference of midday intensities at stations in Europe and South Africa does not exceed 31 per cent. at low altitudes above sea-level and is not greater than 4-8 per cent. at high altitudes must be strongly emphasized. It proves that the general conception of the intensities of the solar radiation in South Africa is, unfortunately quite wrong in that it implies that the radiation as such is many times as intense as it is in Europe.

3. *The length of the days in South Africa and Europe.*

The length of the days in mid-winter and mid-summer for some representative places in South Africa and Europe are presented in Fig. 2. The following places were selected for this comparison: Johannesburg and Capetown, and Davos and Sussex. The time between sunrise and sunset is represented by the respective lengths of the blocks in Fig. 2. The length of the days at the other South African stations (except Messina) is intermediate between Johannesburg and Cape Town; in the same way the length of the days in Bad Nauheim is intermediate between the two European examples given in Fig. 2.

Conclusion: Fig. 2 shows that in summer the days are shorter in South Africa than in Europe, whereas the opposite holds in winter. It also shows that the difference between the length of the South African summer and winter day is smaller than the difference between the summer and winter day in Europe.

4. *Number of hours with bright sunshine in South Africa and Europe.*

One of the most important factors influencing the amount of radiation obtained in various places is the cloudiness. Table 2 gives a comparison of the number of hours of bright sunshine for various places in the Union, in Switzerland, in Germany and in the home counties of two of the cattle breeds imported into South Africa, Sussex and Aberdeen.

TABLE 2.—*Number of bright sunshine hours in South Africa and Europe.*

	EUROPE.				SOUTH AFRICA.			
	Aberdeen and Perth. (Mean Value).	Tun-bridge Wells.	Davos.	Bad Nauheim	Inland.	Cape.	Durban.	
April.........	4·8	5·2	4·8	4·9	9·4	8·5	4·8	October.
May..........	5·5	6·9	5·9	6·7	9·7	9·3	4·5	November.
June.........	6·3	7·1	5·7	6·7	9·8	10·2	4·7	December.
July..........	5·3	6·8	6·4	6·4	8·9	9·7	5·4	January.
August.......	4·6	6·4	6·6	5·8	8·8	8·6	5·9	February.
September....	4·2	5·4	5·5	4·1	8·6	8·1	6·1	March.
October.......	2·9	3·6	4·4	2·7	8·8	7·2	6·5	April.
November....	1·9	2·2	3·2	1·3	8·7	6·5	6·5	May.
December.....	1·1	1·4	2·4	0·7	8·7	6·6	6·6	June.
January......	1·45	1·7	2·7	1·4	8·8	6·5	6·3	July.
February.....	2·4	2·7	4·1	2·2	9·5	6·9	6·3	August.
March........	3·5	4·0	4·7	3·0	9·3	7·0	5·6	September.
MEAN........	3·7	4·5	4·5	3·8	9·1	7·9	5·8	MEAN.

The figures in Table 2 are very striking, because they show a very marked difference between the conditions in South Africa and Europe; the figures speak for themselves. They partly give the explanation why the *total* amount of solar energy can be much greater in some parts of South Africa than in Europe.

Conclusion: The European stations show little over four hours of bright sunshine on an average, in some South African localities the amount is twice as great.

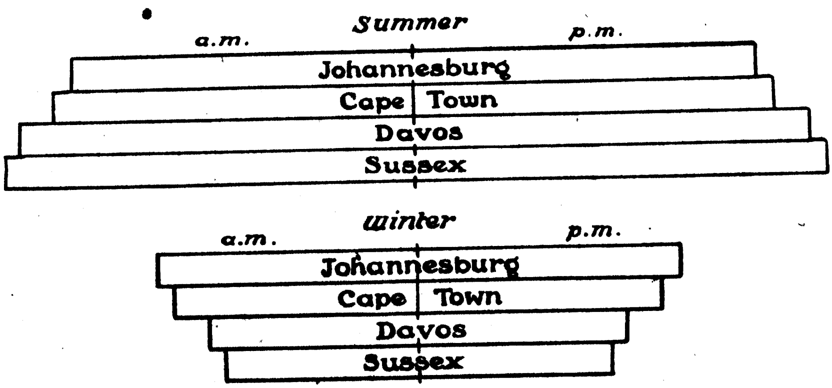


Fig. 2.. Length of Days in S.A. and Europe.

5. *Total amount of sun and sky radiation impinging during various months in South Africa and in Europe.*

Until now intensities have been considered which were measured perpendicular to the incidence of the rays. In the following paragraph the total radiation incident on a *horizontal* surface, including sky radiation, will be discussed. The figures are given in *Kilogram* calories (Cals) per square centimetre.

The South African readings which were obtained at various stations and during different years were combined and mean values for the following groups are given:—

1. Inland stations, including results from Johannesburg (1 year), Bloemfontein (1 year), Onderstepoort (2 years), Armoedsvlakte (2 years).
2. Southern Cape, including results from Cape Town (1 year), Stellenbosch (1 year), Port Elizabeth (1 year).
3. Durban (1 year).
4. Messina (1 year).

The deviation from the mean values given below were not more than 5 per cent. from the average at the inland stations for the summer or winter half year, but amounted usually up to 20 per cent. in a single month and in two cases even up to 27 and 30 per cent. In the Cape the means deviated by 6 per cent. from the summer and winter means; during single months the deviation was nearly the same as at the inland stations.

Continuous records of the total amount of sun and sky radiation impinging on a horizontal surface have, unfortunately, never been published for any place in the British Isles. It is therefore necessary to consider whether an approximate estimate can be obtained from readings recorded elsewhere. For this purpose data published for Bad Nauheim seem most suitable for the following reasons: The centre of the area for Sussex cattle lies at about the same latitude as Bad Nauheim (51°08'N and 50°22'N respectively) giving practically the same angle of incidence of the rays on a horizontal surface in both places. Furthermore, both places are situated at about the same altitude above sea-level, which means an approximately equal thickness of air layer above the two localities. The number of hours with bright sunshine is on an average 3·8 hours per day in Bad Nauheim, and 4·5 hours per day in Tunbridge Wells, Sussex, i.e. 15 per cent. more for the latter. This figure should be kept in mind when considering the radiation conditions in the home country of the Sussex breed. For the comparison of the South African with the European data, the radiation intensities for Bad Nauheim will be used.

The comparison of South African and European data on solar radiation is presented in Fig. 3, in which 3a gives a comparison of 4 South African stations, and 3b shows the data of the South African inland stations as compared with Davos and Bad Nauheim. These graphs at once show two outstanding facts, namely—

1. that the difference between the intensities measured at various widely separated localities in the Union is not great, and
2. that a comparison with European conditions shows a marked drop of itensities during the winter months in Europe, which is not so pronounced in the Union of South Africa.

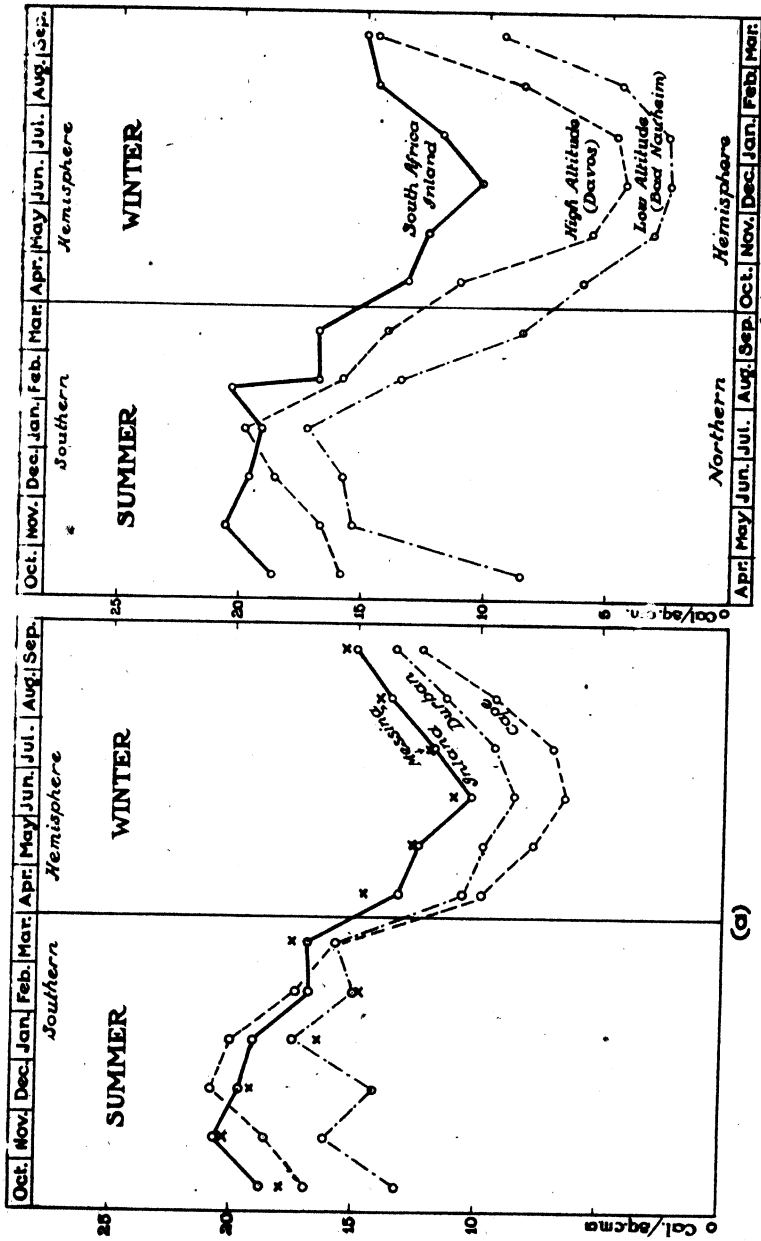


Fig. 3: Monthly total amounts of sun and sky radiation.
(a) at various stations in South Africa.
(b) at 2 stations in Europe as compared with South African inland stations.

A much closer study of the conditions is facilitated by the figures in Table 3, which gives the monthly totals obtained by the Solar Radiation Survey in the Union and the corresponding data for Davos and Bad Nauheim.

TABLE 3.—*Average monthly totals of sun and sky radiation, impinging on a horizontal surface, given in Kilogram calories per square centimetre.*

	SUMMER HALF YEAR.						WINTER HALF YEAR.					
Month.......	Oct.	Nov.	Dec.	Jan.	Feb.	March.	April.	May.	June.	July.	Aug.	Sept.
Cape........	16·9	18·6	20·8	20·0	17·3	15·7	9·8	7·7	6·4	6·9	9·3	12·3
Durban......	13·2	16·2	14·2	17·5	15·0	15·7	10·6	9·8	8·6	9·3	11·3	13·4
Messina......	17·9	20·3	19·2	16·5	14·8	17·5	14·6	12·6	11·0	11·9	14·0	15·5
Inland.......	18·7	20·5	19·7	19·1	16·8	16·8	13·2	12·4	10·2	11·8	13·6	15·0
Davos.......	15·8	16·7	18·6	19·8	15·8	14·0	11·1	5·7	4·4	4·8	8·6	14·6
Bad. Nauheim	8·5	15·4	15·8	17·2	13·4	8·6	6·0	2·2	1·6	1·7	3·6	9·4
Month.......	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.

Conclusion: The difference between the total amount of radiation in South Africa and Europe during the *summer* half year is comparatively small. During the 16 hours of a long European mid-summer day the total amount of incident radiation is even greater at Davos than in most places in the Union. At low altitudes, represented by Bad Nauheim, the amount of energy reaches nearly that recorded at Durban. During the *winter* half year the difference of the amount of radiation is considerable at high altitudes. Davos then obtains only a little more than half the amount registered at the South African Inland stations. At a low altitude this difference is even more marked. During the two mid-winter months, for instance, the amount of radiation is about five times bigger at Durban than at Bad Nauheim.

These facts are important and will therefore be more emphasized by the figures in Table 4, which present the totals for the half years.

TABLE 4.—*Comparison of half yearly and yearly total amounts of radiation incident in South Africa and in Europe.*

	Summer Halfyear.	*Winter Halfyear.*	*Total Year.*
Cape..........................	109·4	52·4	161·8
Durban..........................	91·8	63·0	154·8
Messina..........................	106·2	79·6	185·8
Inland..........................	111·5	75·8	187·3
Davos..........................	100·9	49·2	150·1
Bad Nauheim..........................	78·9	24·5	103·4

The figures in Table 4 show that during the summer half year at Bad Nauheim 79 Cals (or 70 per cent.) were recorded, as compared with 112 Cals (100 per cent.) recorded at the South African inland stations. In winter

this difference is much bigger, Bad Nauheim recording only 32 per cent. of the amount registered at the South African inland stations. The totals for the year show 103 Cals. at the former, and 187 Cals. at the latter station.

This clearly demonstrates that *the differences in the annual totals are due predominantly to the differences during the winter half year* and less so to those during the summer half year. If this is so, the question might be asked whether the much greater total annual amount of radiation is really of such significance? If it were proved that too much solar radiation had a harmful influence on cattle, would not everybody expect this influence to take place particularly during the hot summer months rather than during the cooler winter, when the solar radiation is a pleasantly warming factor.

Summarizing, the comparison between the South African and European stations shows the following:—

1. *Angle of incidence of the solar rays.* Distinctly larger in South Africa.
2. *Midday intensities.* At Johannesburg nearly equal to those at Davos, but at Durban on an average higher than at Kew.
3. *Length of days.* Days in South Africa shorter during the summer, but longer during winter than in Europe.
4. *Number of hours with bright sunshine.* South Africa many more hours with bright sunshine during the whole year, but particularly during winter.
5. *Monthly total amount of sun and sky radiation incident on horizontal surface.* In summer equal or slightly greater amounts in South Africa, in winter amounts much larger here than at the European stations.
6. *Yearly total.* Much greater amount at the South African inland stations (187 $Cals/cm^2$) than at the lowlands of Europe (103 Cal/cm^2).

Before we turn to the discussion of how much solar radiation is incident on the surface of cattle in South Africa and Europe it seems desirable to point out the possibility that two factors may cause a stronger influence of the radiation here than in Europe:—

1. The exposure to comparatively great amounts of radiation *throughout the year.*
2. The simultaneous influence of other climatic factors, such as air temperature, humidity and wind.

These three latter factors play an important rôle with regard to the possibility of losing heat against the environment (see page 350). A discussion on this point does not lie within the scope of this article, but it is of interest to give an indication of the conditions prevailing in South Africa and Europe. The cooling effect of the air depends largely on its movement, because in absolute calmness this effect is comparatively small. In the open, however, the air is seldom absolutely calm; its temperature is then one of the factors governing its cooling effect. The difference between normal body temperature (102° F.) and the maximum average air temperature for various localities in South Africa and Europe is presented in Table 5.

TABLE 5.

Difference between body (102° F.) and maximum air temperature (in degrees F.).

Stations.	Summer.						Winter.						Yr.
	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	
Cape Town	31	28	24	**22**	**22**	24	28	33	**36**	**37**	**36**	35	30
Durban	27	25	23	**21**	**21**	23	24	27	30	**31**	**31**	30	26
Messina	13	12	**11**	**11**	13	14	17	21	**25**	**27**	23	18	17
Inland	20	20	**17**	**17**	19	22	26	32	**37**	**37**	32	26	25
Kew	49	42	37	**33**	**33**	37	45	53	56	**57**	**57**	54	46
Aberdeen	54	49	43	**40**	**40**	44	50	56	**59**	**59**	**59**	57	51
Davos	46	38	31	**28**	**30**	35	41	52	**61**	**62**	60	54	45
	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Yr.

NOTE.—The bold figures indicate the two months during which the difference between maximum air temperature and body temperature is smallest in summer and largest in winter.

Conclusion.—During the two *hottest* months of the year the average maximum air temperature at the South African stations is 11 to 23° F., at the European stations 33 to 44° F. below normal body temperature of cattle. During the *coldest* months of the year the difference between average maximum air temperature and body temperature is 25 to 37° F. at the South African, and 57 to 59° F. at the European stations.

PART II.

THE AMOUNT OF RADIATION ABSORBED ON THE BODY SURFACE OF CATTLE IN SOUTH AFRICA AND EUROPE.

The factors which determine the amount of radiation absorbed by the body surface of cattle change continually, from hour to hour as well as from day to day. Firstly there is the intensity of the incident radiation which depends largely on the amount of clouds present, and which varies with the altitude of the sun, with the thickness of the air layer and its composition. Secondly, with a given intensity of the solar beam, the amount which is actually absorbed depends on the colour and the nature of the surface of the animal, as well as on its size. Lastly, there is the animal itself which continually changes its position and by doing so exposes its side or its front to the incoming beam of solar radiation. The large number of variables make a calculation of their combined effect very involved and it is consequently essential to restrict our discussion to a few clearly defined examples.

This discussion will be confined to clear days only because they represent extreme conditions of insolation. Mid-summer and mid-winter readings are selected to represent the highest possible amount of radiation incident during a clear, long mid-summer day and a short mid-winter day, with high and low sun altitudes respectively. With regard to the colour of the hides, the discussion is limited to brown hides only, since brown is one of the most

common colours of cattle in South Africa. Preliminary measurements on hides of different colours have shown that the absorption varies a great deal, and detailed investigations on this subject are still in progress. With regard to the posture of the animal two extreme cases are considered, firstly when the animal is kept at right angles to the sun's rays, and secondly when it is facing the sun. These two cases represent conditions under which the animal is exposed to the smallest and largest possible amount of radiation respectively. In any other position the animal must obtain an amount which lies between these two extremes.

To repeat: The following discussion will include clear days only, in mid-summer and mid-winter, the solar rays striking the animal perpendicularly or parallel to its sagittal axis. *It is realized that, although the calculations are based on accurately determined figures, the combination of them can only give an approximate estimate of the true conditions.*

The *total amount of radiation* which is absorbed by the hairy coat of cattle depends firstly on the amount of incoming radiation and secondly on how much of this incoming radiation is absorbed.

The incoming radiation consists of—

(*a*) the amount of direct solar radiation;

(*b*) the amount of scattered radiation from the sky;

(*c*) the amount of radiation reflected from the ground.

How much of the incoming radiation is absorbed depends on the following factors:—

(*a*) the size of the animal, which determines the area exposed to radiation; the amount absorbed is determined by the cross section which the animal presents to the incoming *direct* solar radiation, and by the total curved area for *sky* radiation and radiation *reflected from the ground*;

(*b*) the absorption coefficient of the hairy coat, i.e., the percentage of the incident radiation which is absorbed. This absorption coefficient varies with the angle of incidence of the rays on the various parts of the body.

Before one can discuss the combined influences of all these factors, it is again essential, as in Part I of this paper, to deal with the various factors separately.

The stations under consideration in this comparison are Johannesburg (S.A.), Davos (Switzerland) and Potsdam (Germany). The latter station will replace Bad Nauheim in the following discussion, because data for Bad Nauheim suitable for a comparison, are not available and Potsdam represents approximately similar conditions. (For geographic data of the above-mentioned places see page 328.

1. *The amount of direct solar radiation.*

Table 6 gives mean values of the direct solar radiation (measured perpendicularly to the solar beam) for clear days, related to the sun's altitude.

TABLE 6.

Intensity of direct solar radiation on clear days in mid-summer and mid-winter related to sun heights in gram calories per square centimetre per minute (from "Tabellen, etc.", 1938).

	MID-SUMMER.									
Sun's altitude	5°	10°	15°	20°	30°	40°	50°	60°	70°	80°
Johannesburg	0·52	0·83	1·01	1·14	1·33	1·44	1·51	1·55	1·56	1·58
Davos	—	—	—	0·89	1·25	1·33	1·38	1·42	—	—
Potsdam	0·27	0·53	0·73	0·85	1·03	1·14	1·21	1·26	—	—
	MID-WINTER.									
Sun's altitude	5°	10°	15°	20°	30°	40°	50°	60°	70°	80°
Johannesburg	0·35	0·60	0·81	0·98	1·22	1·35	—	—	—	—
Davos	—	1·07	1·26	1·37	—	—	—	—	—	—
Potsdam	0·52	0·82	1·00	—	—	—	—	—	—	—

2. *The amount of scattered radiation from the sky.*

This was frequently measured during the period when the South African Solar Radiation Survey was carried out. The ratio between sun and sky radiation (total per day) was found to be approximately 11:1 on clear summer days and 8:1 on clear winter days. An average ratio of 10:1 was used for the Johannesburg calculations. For Europe very few data are available. From readings published by Dorno (1927) it seems justified to assume an average ratio of 10:1 for Davos and of 4:1 for Potsdam.

3. *The amount of radiation reflected from the ground.*

Of the total amount of radiation incident on a horizontal surface on a cloudless day a certain amount is reflected from the ground. This amount depends on the nature of the surface of the ground. Measurements carried out at Onderstepoort at a sun's altitude of about 30° showed readings similar to those published by various authors (see Büttner, 1938), namely:—

The reflection from a surface of green short grass was found to be 30 per cent. of the total incoming radiation.

The reflection from light brown dry grass amounted to 28 per cent., and the reflection from a greyish sandy road was 26 per cent.

For the following calculation a mean value of 30 per cent. reflection of the total incoming radiation by the ground was assumed.

4. *The body surface area and how much of it is influenced by the direct solar, the sky radiation and the radiation reflected from the ground.*

(a) *The body surface area and direct solar radiation.*

The amount of direct radiation incident on the animal at various sun heights can be estimated by multiplying the area of the shadow which it casts on a horizontal surface by the intensity of the direct radiation, also measured on a horizontal surface.

The size of the shadow of the animal varies with the height of the sun and had, therefore, to be determined at various altitudes of the sun. The animal used was a full grown Sussex bull, 3 years old, 1,760 lb. in weight. The shadow areas are given in the form of a graph in Fig. 4. The abscissa represents the sun altitudes at which drawings of the bull's shadow were made, the ordinate indicates the size of the shadow in square centimetres. The shadows were measured on a horizontal plane except for sun altitudes lower than 15° for which the projection on a vertical plane was used. Graph A in Fig. 4 represents the bull standing perpendicularly to the direction of the sun's beam, graph B shows the shadow areas when the bull was facing the sun, i.e., the greatest and smallest possible shadow area at each particular sun altitude.

(b) The body surface area influenced by the sky radiation.

The total surface area of the bull was obtained from measurements of hides from 3 bulls similar in size and weight to the one which was used for the determination of shadow areas.* The average total surface area was 75 square feet or 65,000 square centimetres. This figure was verified by applying Hogan's (1923) formula to the bull used in this experiment. The surface area calculated is 64,500 sq. cm. The measured value of 65,000 sq. cm. was used. The contribution of the sky radiation should be halved because the animal is not completely surrounded by sky but receives sky radiation from above only. This amounts to the same as using half the surface area of the bull for the calculation of the sky radiation and half the area for the radiation reflected from the ground. For the calculation of the sky radiation, therefore, half the total surface area or 32,500 sq. cm. were used.

(c) The body surface area influenced by the radiation reflected from the ground.

The same considerations as mentioned under (*b*) refer to the radiation reflected from the ground on to the animal, the area being also approximately 32,500 square centimetres.

5. The absorption of the radiation by the hairy coat of brown cattle.

(a) The absorption of direct solar radiation.

The absorption of direct solar radiation on the hairy coat of cattle was determined by measuring the amount of radiation reflected from hides. The use of hides was necessary, because a living animal is not large enough to cover the whole area from which the instrument receives radiation. The measurements had, therefore, to be carried out on hides spread out level on a large board. In order to ascertain whether one is entitled to apply readings obtained from a dead hide to a living animal the following experiment was carried out:—

On a cloudless day the bull was placed in a crush so that he could not alter his position with regard to the incoming rays or to the background before which he was standing. Within a few minutes (practically no change in intensity or direction of the sun's rays having taken place) the total incoming radiation and the radiation reflected from the bull was measured.

* These measurements were kindly placed at my disposal by Prof. J. H. R. Bisschop.

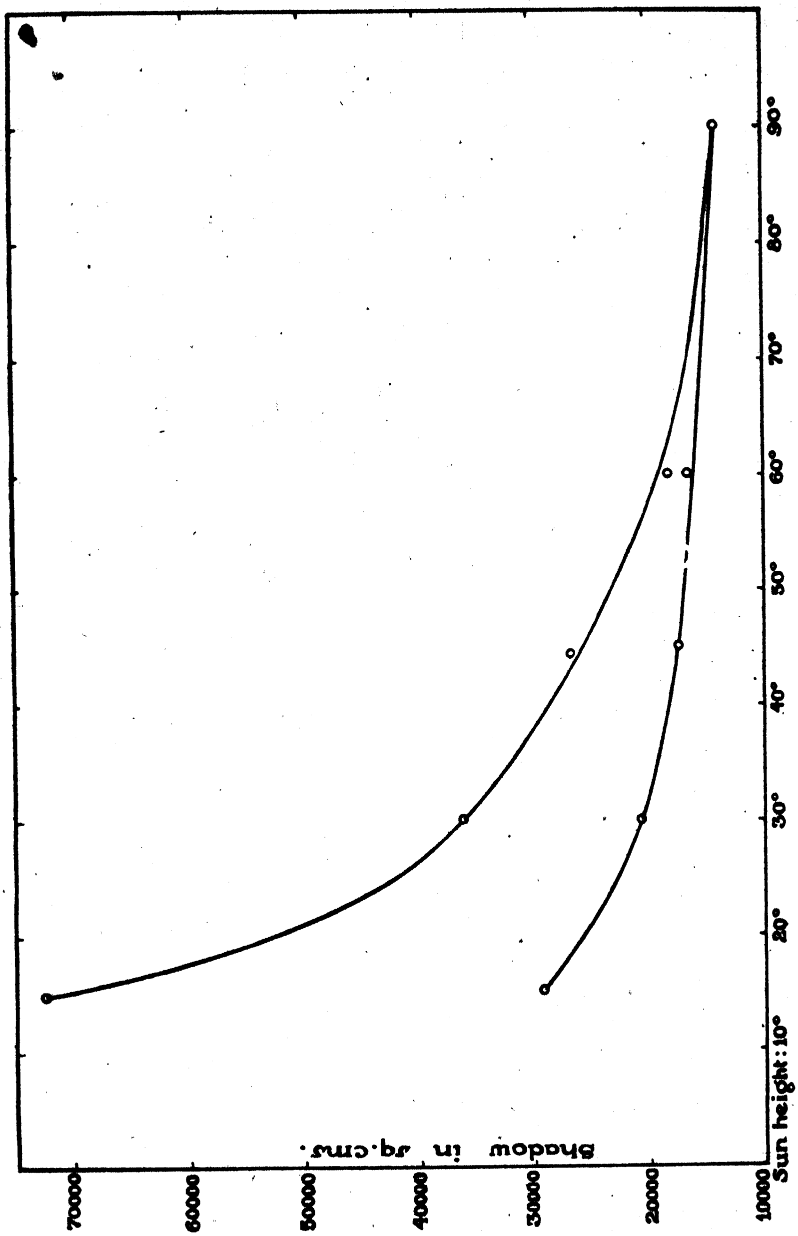


Fig. 4: Size of shadow of a bull measured at various altitudes of the sun

Immediately afterwards the bull was covered with a dead hide, which was tied round his body so that the surface presented the same shape as the animal's body. The incoming and reflected radiation were then again determined. The results obtained were the following:—

	Absorption.				
Sussex bull, dark brown	81%	87%	80%	80%	87%
Sussex hide, dark brown	79%	87%	—	—	—
Red Poll hide, lighter brown ...	—	—	79%	78%	86%

These readings are not absolute, but only relative values which permit us to compare results obtained from a living animal and a dead hide. The readings show that the absorption in both is equal (within the limit of the instrumental error). This proves:—

1. That the absorption on the hairy coat of cattle does *not* depend on the state of the underlying skin.
2. That the absorption is determined only by the absorbing power of the hairy coat.
3. That the difference in the hairy coat of a living animal or a dead hide (with regard to dustiness, smoothness and glossiness) results in minute differences in absorption if the colour in both cases is similar.

To determine whether a change in functional activity of a living animal results in a difference between animal and hide readings, measurements were carried out on the bull after he had a hard fight with another bull. He was panting heavily (at a low air temperature) which shows that he was on a higher level of functional activity than usually. The absorption was found to be equal, i.e. 87 per cent. on the bull and on the hide.

The reflection from cattle hides was determined with a thermopile at various angles of incidence of the solar beam. Owing to the fact that the reflecting power of a surface depends on its colour and the structure of its surface, it can be assumed that different hides might show different absorption coefficients. The present investigation was restricted to one example, namely the absorption on brown hides of different structure. A hide of a Sussex × Afrikaner ox and another one from a high grade Afrikaner cow were used. The colour of the two hides was similar, the structure of the hairy coat distinctly smoother and the hairs more glossy in the case of the high-grade Afrikaner. The Sussex × Afrikaner hide had been dried, the other hide was used immediately after slaughtering without being washed or dried to represent as closely as possible the hide of a living animal.

The influence of the direction of the hair on the absorption of the incident rays was also taken into consideration. Reflection readings were taken when the hairs were pointing towards the sun (or to be more precise in the azimuthal plane of the sun), away from the sun or at right angles to the incoming rays. The absorption coefficients obtained from these measurements are given in Fig. 5, where the circles refer to the Sussex × Afrikaner hide, the dots to the high-grade Afrikaner hide.

Conclusion.—As will be seen from the graph in Fig. 5 there is only a small difference in absorption between the Afrikaner × Sussex hide and the high-grade Afrikaner hide. The absorption on the high grade Afrikaner hide is only about 2-4 per cent. less, due to the difference in structure, glossiness and colour. The direction of the hairs with regard to the incoming rays results in a very small difference of absorption at great altitudes of the sun. Only at low solar altitudes is the absorption distinctly higher when the hairs are pointing towards the sun. Below an altitude of 30° the absorption decreases more rapidly, but nevertheless at 10° it still amounts to 50 per cent., whether the hair is pointing towards or away from the sun.

An average absorption coefficient was estimated by calculating the contribution which various areas corresponding to different angles of incidence make to the total absorption. To facilitate the estimation of this contribution the bull was regarded

firstly as a right circular cylinder (for the beef type of cattle) and secondly as a cylinder of elliptical cross section (milk type), the height of the ellipse being one and a half times its width. The figures representing the contributions of the various areas were multiplied by the corresponding absorption coefficients and a weighted average was so obtained. The values were: for the circular section 80 per cent., and for the elliptical section 78 per cent. which suggests that the difference in shape of a circular or an elliptical cross section does not greatly influence the mean absorption coefficient. A value of 80 per cent. mean absorption was assumed in the following calculations.

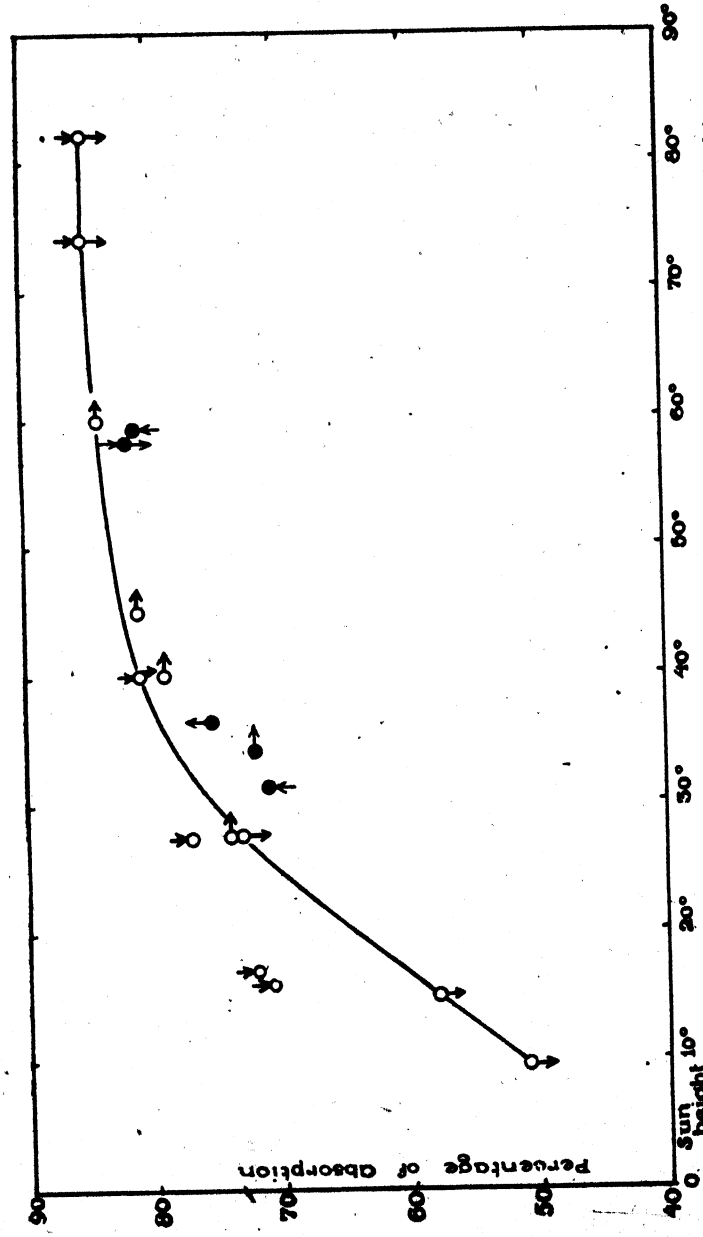


Fig 5: Absorption coefficient of direct solar radiation measured on a hide with hair pointing :-
away from sun. towards sun. at angle of 90°.
O = Sussex x Afrikaner ● = High grade Afrikaner

(b) The absorption of the sky radiation.

The reflection of the sky radiation from the horizontally spread hide was determined at various sun altitudes, by measuring the reflection when the hide was shaded against direct solar radiation. The absorption was found to be approximately 72 per cent., irrespective of the altitude of the sun.

(c) The absorption of the radiation reflected from the ground.

Since the radiation reflected from the ground is similar in composition to the direct solar radiation in that it is composed of all wavelengths, the figure of 80 per cent. reflection is used.

From all the above-mentioned data it is possible to calculate the approximate amount of radiation which is actually absorbed by the surface of cattle under certain conditions. Before this calculation is presented it seems desirable, however, to summarize shortly the various items which have been used for this calculation.

Items:	*Figures used:*
1. The intensity of the direct solar beam.	See Table 6, page 340.
2. The intensity of the scattered radiation from the sky.	1/10 of the direct solar radiation at Johannesburg and Davos, ¼ of the solar radiation at Potsdam.
3. The intensity of the radiation reflected from the ground.	This was measured and found to be 30 per cent. of the total sun and sky radiation.
4. The body surface area influenced by direct solar radiation.	The shadow of a bull was measured at various altitudes of the sun, see Fig. 4, page 342.
5. The body surface area influenced by sky radiation.	Half the total surface area, i.e. 32,500 sq. cm. were used.
6. The body surface area influenced by the radiation reflected from the ground.	As under No. 5.
7. Absorption co-efficient of direct solar radiation at various angles of incidence of the rays on brown hides when spread over a level surface.	This was measured on hides of two different breeds of cattle; the results are given in Fig. 5, page 344.
8 Mean absorption coefficient of direct solar radiation considering the contribution of various areas with regard to the angle of incidence on them.	The weighted average of absorption on cattle was estimated to be 80 per cent. of the incoming direct solar radiation.
9. The absorption of sky radiation.	This was measured and found to be approximately 72 per cent. of the incoming sky radiation.
10. The absorption of radiation reflected from the ground.	This was estimated to be 80 per cent. of the radiation reflected from the ground (see under No. 3).

N.B.—It will be remembered that the intensities of cloudless mid-summer and cloudless mid-winter days have been used for the calculations and that it was assumed that the animal was either facing the sun (parallel) or standing at a right angle to the incoming solar beam (perpendicular).

TABLE 7.

Calculation of the Total Amount of Direct Solar Radiation Absorbed on the Body Surface of a Bull.

True (Solar) Time.	Sun's Altitude.	Intensity of Direct Solar Radiation. (cals./min.)	Intensity × Sinus Sun Height (*h*).	RIGHT ANGLE.				PARALLEL.			
				Shadow Area in sq. cm.	Shadow Area × Sinus (*h*).	Solar Radiation *Incident* on Body Surface in cals./min.	Solar Radiation *Absorbed.* (80 % of the incident radiation.)	Shadow Area in sq. cm.	Shadow Area × Sinus (*h*).	Solar Radiation *Incident* on Body Surface in cals./min.	Solar Radiation *Absorbed.* (80 % of the incident radiation.)
JOHANNESBURG SUMMER-DAY.											
5·35	5°	0·52	—	—	(19,500)	10,100	8,100	—	—	—	—
5·52	10°	0·83	—	—	(19,100)	15,900	12,700	33,900	5,900	4,900	3,900
6·21	15°	1·01	—	72,400	18,750	18,900	15,100	28,600	7,400	7,500	6,000
6·45	20°	1·14	—	53,300	18,200	20,800	16,600	24,800	8,500	9,700	7,800
7·30	30°	1·33	—	36,300	18,100	24,100	19,300	20,100	10,100	13,400	10,700
8·18	40°	1·44	0·93	28,800	—	26,800	21,400	18,100	—	16,800	13,400
8·40	45°	1·48	1·05	25,900	—	27,200	21,800	17,200	—	18,100	14,500
9·04	50°	1·51	1·16	23,100	—	26,800	21,400	16,800	—	19,500	15,600
9·48	60°	1·55	1·34	18,500	—	24,800	19,800	15,900	—	213,00	17,000
10·34	70°	1·56	1·47	16,000	—	23,500	18,800	15,000	—	22,100	17,700
11·18	80°	1·58	1·56	14,600	—	22,800	18,200	14,300	—	22,300	17,800
12·00	90°	1·58	1·58	14,000	—	22,100	17,700	14,000	—	22,100	17,700
JOHANNESBURG WINTER-DAY.											
7·15	5°	0·35	—	—	(19,500)	6,800	5,400	—	—	—	—
7·40	10°	0·60	—	—	(19,100)	11,500	9,200	—	5,900	3,500	2,800
8·07	15°	0·81	—	—	18,750	15,200	12,200	—	7,400	6,000	4,800
8·35	20°	0·98	—	—	18,200	17,800	14,200	—	8,500	8,300	6,600
9·45	30°	1·22	—	—	18,100	22.100	17,700	—	10,100	12,300	9,800
11·35	40°	1·35	0·87	28,800	—	25,000	20,000	18,100	—	15,700	12,600

6. *The total amount of radiation which is absorbed on the body surface of cattle under South African and European conditions.*

From the above items the amount of direct solar radiation absorbed on the surface of brown cattle was calculated for various altitudes of the sun for three different localities, Johannesburg, Davos and Potsdam. An example of this calculation is given for Johannesburg in Table 7. The distribution of the amount of absorbed radiation over half a mid-summer and mid-winter day is given in Figure 6. In each of the six sub-divisions two graphs are represented: the higher one indicates the total amount of direct solar radiation absorbed when the animal is standing *at right angles* to the rays, the lower graph gives the amount absorbed when the animal is *facing* the sun. The total area included under each graph represents the total amount absorbed during half a day, twice that amount gives the daily total. The respective figures are given in Table 8, column (a). To these figures have to be added the amount of absorbed radiation from the sky [Table 8, column (b)] and of the radiation reflected from the ground [column (c)]. *The total amount of radiation absorbed by the hairy coat of a bull during the whole day* is given in the last two columns of Table 8.

TABLE 8.

Amount of energy absorbed from direct solar radiation (a), sky radiation (b), radiation reflected from the ground (c), and their totals (d).

	(a)		(b)	(c)	(d)	
	Direct Solar Radiation.		Sky Radiation.	Radiation Reflected from the Ground.	Total Amount of Radiation Absorbed per Day.	
	Perpendicular.	Parallel.			Perpendicular.	Parallel.
SUMMER.						
Johannesburg..	14·700	10·400	1·700	6·300	22·700	18·400
Davos.........	14·300	8·000	1·600	5·900	21·800	15·500
Potsdam.......	13·700	8·600	3·500	5·300	22·500	17·400
WINTER.						
Johannesburg..	9·300	4·900	0·800	2·900	13·000	8·600
Davos.........	8·300	3·100	0·300	1·200	9·800	4·600
Potsdam.......	4·500	1·500	0·400	0·600	5·500	2·500

The total amount of radiation absorbed during a whole mid-summer day on the body surface of a bull is strikingly great, namely more than 20,000 *Kilogram calories* (Cals.). Cattle of smaller size will, of course, absorb less, because their surface area is smaller. An average surface area of cattle of 990-1,200 lb. live weight is 51,000 sq. cm. (Hogan. 1923) as compared with 65,000 sq. cm. of the bull under discussion. The amount of radiation absorbed during a day will be smaller in the same proportion, assuming that

the shape of different cattle is fairly similar. Consequently the amount of radiation absorbed by cattle of average size will be reduced by the ratio of the surface areas, i.e., 65,000:51,000 or 1:0·78. Instead of more than 20,000 Cals. being absorbed by the bull, smaller cattle will absorb approximately 17,000 Cals.

These quantities can perhaps best be imagined if one considers that 20,000 Cals. would suffice to heat 44 gallons of water from 0° C. to boiling point. The same amount would also suffice to increase the body temperature of the bull from normal body temperature to approximately 145° F. if heat loss were prevented. (This figure of 145° F. represents the lower limit and was arrived at by considering that 80-90 per cent. of the body consists of water with a specific heat of 1·0, and that the specific heat of the remaining parts of the body cannot be higher than unity.)

The importance of the great amount of heat absorbed by the hairy coat of cattle is also shown by comparing it with the amount of heat produced by metabolism. According to Forbes (1928) the heat production of a bull of 1,000 lb. live weight on mixed rations amounts to 9,600 Cals. per day. *During the 15 daylight hours of a mid-summer day the heat produced is, therefore, approximately 6,000 Cals., whilst 17,000 Cals. are absorbed on the surface of the body.* This fact emphasises the important rôle which solar radiation plays amongst the environmental influences on cattle. The loss of heat must necessarily be rendered more difficult if, during a day, the body surface absorbs about three times as much heat from radiation as is produced by the metabolism.

The different ways in which heat can be lost are the following:—

1. Loss of heat through long wave radiation, which can only take place against an environment at a lower temperature than body temperature. The greatest loss of heat takes place outwards against space. Heat is also lost by long wave radiation against objects in the vicinity of the animal, but if their temperature is higher than body temperature, heat is even gained, as for instance from the ground.

2. Loss of heat by evaporation of water, i.e., evaporation of free water from the surface of the body, the respiratory organs, and expulsion of heated water particles during expiration. These are purely physiological processes and will, therefore, not be discussed here. It may be mentioned, however, that humidity of the air influences the rate of heat loss by evaporation of water.

3. The loss of heat through conduction and convection, the rate of which is influenced by the air temperature and the wind. Air in the immediate neighbourhood of the body is heated up by the heat from the body and by convection, is replaced by cooler air. This convection takes place even if the air is absolutely calm. That wind accelerates this process is easy to understand. The significance of the figures of the difference between body and air temperature (given in Table 5, page 338) becomes immediately apparent.

From the above it can be realized that the means of losing heat are limited. An extra amount of 17-22,000 Cals. absorbed by the hairy coat from radiation must be very important in the heat regulating mechanism.

After discussing the significance of the figures in Table 8, the remaining points of interest in this table may shortly be summarized as follows:—

The total amount of radiation absorbed on the surface of a bull during a *mid-summer* day is similar, whether the animal is exposed under highveld conditions in South Africa, in the alpine regions of Switzerland or under lowland conditions in Central Europe. It is important to realize, however, that this statement does not refer to the general radiation *climate* in which the animals live in these three localities, because clear days only were used as a basis for the above calculations.

In *mid-winter*, when the difference in the angles of incidence of the rays, the duration of exposure, etc., play a bigger part in the total amount of radiation absorbed, the differences between the three localities are much more pronounced. In other words, the difference between the localities influences the mid-winter amounts of radiation more than the mid-summer amounts. In Johannesburg in mid-winter a bull would absorb about half of what it absorbs in mid-summer, in Davos a little less than half, in Potsdam only a quarter of the amount absorbed during a summer day.

The *position of the animal* with regard to the incoming solar beam influences the amount of absorbed radiation only to a small extent during a mid-summer day. In mid-winter this difference is distinctly greater. When facing the sun the animal absorbs only about half the amount of what it would absorb when standing perpendicularly to the solar beam.

7. *The reduction of the amount of radiation by natural and artificial shade.*

As pointed out above, the amount of radiation absorbed under Johannesburg conditions is about equal (summer) or greater (winter) than that absorbed under European conditions. On the other hand the difference between maximum air and body temperature at Johannesburg, for instance, and more so at Messina, is a fraction only of what it is in Davos or Potsdam. It is, of course, impossible to alter the temperature of the air, but the great amount of radiation absorbed on the body surface of cattle can be altered by providing shade. The amount of heat which has to be eliminated is then reduced predominantly to the animal's basal metabolism, except when the air temperature is so extremely high as to exceed body temperature.

To give an approximate estimate of how much of the extra heat absorbed from radiation can be eliminated by natural or artificial shade, measurements of the reducing effect of shades have been carried out at Onderstepoort. The following table contains readings of the total sun and sky radiation measured underneath a large tree, under open thorn bush and under an artificial shade created by a layer of dry branches on top of a frame. The readings were taken for ten cloudless days in each case and compared with values obtained during the same days on an instrument freely exposed to insolation. In Table 9 the total amount of incoming radiation underneath the shades is given as well as the percentage which it represents of the total amount outside the shade.

The effect of even light shading, as was obtained from the artificial shade, can be seen from the above figures. It results in a protection of the animal against 60-70 per cent. of the incoming total radiation. This would reduce the additional heat absorbed from radiation to a considerable extent. The significance of this fact need not be emphasised.

TABLE 9.

Total amount of incoming radiation in natural and artificial shade and their percentage of the radiation obtained in the open.

	Radiation.		Percentage of the Amount in the open.
	Outside.	In Shade.	
			Per cent.
Under large tree	608	233	38
Under thornbush	458	148	38
Under artificial shade	384	147	32

SUMMARY.

Two aspects of solar radiation in its relation to cattle in South Africa and Europe are considered. The first is the solar radiation itself and the factors influencing its intensity and total amount incident on a horizontal surface at various places. The second is the question of how much radiation is absorbed by the body surface of cattle in South Africa and Europe.

A comparison of solar radiation in South Africa and Europe shows the following facts:—

1. The angle of incidence of the solar rays is distinctly larger in South Africa than in Europe.

2. The midday intensities in Davos (Switzerland) are on an average nearly equal to those at Johannesburg (South Africa); at Kew (England) they are on an average lower than at Durban (S.A.).

3. The days are shorter in South Africa during summer, but longer during winter.

4. The number of hours with bright sunshine is much greater in South Africa during the whole year, particularly during winter.

5. The monthly total amount of sun and sky radiation is equal or slightly greater in summer; it is, however, markedly greater during winter in South Africa than in Europe.

6. The yearly total amount of incident radiation is 187 Kilogram Calories per square centimeter at the South African Inland Stations as compared with 103 Cals./sq. cm. on the lowlands of central Europe.

In Part II the total absorption of radiation from the sun, the sky, and that reflected from the ground on to the body surface of cattle under South African and European conditions is calculated. The discussion is limited to a few clearly defined examples. Figures of the amount of solar and sky radiation impinging on to the animal during a clear mid-summer and a clear mid-winter day are given, the animal either standing at right angles to the solar beam or facing the sun. The absorption of the incoming radiation is determined by reflection measurements on two brown bovine hides of different breeds (Sussex × Afrikaner and high grade Afrikaner) and figures of

the absorption of direct solar and sky radiation and the absorption of radiation reflected from the ground are presented. From these data the total amount of radiation absorbed by the body surface of cattle is calculated. This amount is found to be strikingly high, e.g., more than 20,000 Kilogram Calories during a clear mid-summer day, regardless whether the animal is exposed on the highveld of South Africa, in the alpine region of Switzerland or on the lowlands of central Europe.

A comparison of the total amount of radiation absorbed by the hairy coat and the heat produced by metabolism shows that cattle absorb nearly three times as much heat from radiation as they produce by metabolism during an equal period.

The means of losing heat in order to keep their body temperature within safe limits are discussed from a physical point of view. With regard to a possible reduction of the amount of heat which has to be eliminated from the body, the effect of shade on the amount of incident radiation is discussed. Figures of the reduction of the incoming solar radiation by natural and artificial shade are given which show that the amount of heat absorbed by the hairy coat of cattle can, by providing shade, be reduced to 30-40 per cent. of the amount which impinges on to the animal in the open veld.

Acknowledgment.

I wish to thank Mr. J. S. Elder for the valuable help with regard to physical measurements and calculations, Prof. J. H. R. Bisschop for measurements and advice regarding the surface area of cattle and Miss E. Laurence for the drawing of the figures.

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Curve Fitting by the Orthogonal Polynomials of Least Squares.

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1. INTRODUCTION.

THE use of the orthogonal polynomials of least squares as a statistical procedure of curve fitting has become widespread in recent years. It has found applications in many fields of research; its ultimate success in Biology and related subjects will largely depend upon the ability of the biologist to realize the advantages and, at the same time, the limitations that are inherent in this procedure. Unfortunately most of the theory of polynomial fitting is available only in a highly technical form.

It is therefore intended to present in a series of papers, of which this is the first one, the implications of orthogonal polynomial fitting. At the same time, the best practical procedures will be given.

In this paper the systems of orthogonal polynomials mainly used in practice are derived from a common general formula, which is established by the principle of least squares, utilizing results from the Finite Calculus. The Aitken–Chebyshev orthogonal polynomial is recommended for practical use, and, due to a simplification, the fitting process, by means of an extensive set of appended tables, becomes very easy in practice.

At the same time these papers must be regarded as forming the background for a series of papers, appearing in the near future, on the problem of Bio-climatology.

Since it is attempted to present a more or less selfsufficient paper, the non-statistical reader, for whom it is actually intended, should have a fair amount of success, if it be kept in mind that a statistical paper should be *worked* through.

2. Mathematical Introduction.

(a) *Polynomial Interpolation.*

The name *polynomial* (Gr. *polys*, many, L. *nomen*, a name) is given to an algebraical function to express the fact that it is constituted of a number of terms containing different powers of x, connected by the signs $+$ or $-$, i.e., an expression of the form

$$C_0 + C_1x + C_2x^2 + \ldots + C_r x^r.$$

The general problem of interpolation consists in representing a function, known or unknown, in a form chosen in advance with the aid of given values, which this function takes for definite values of the independent variable.

Weierstrass first enunciated the theorem that an arbitrary function can be represented by a polynomial with any assigned degree of accuracy. For a mathematical discussion of the theory of approximation *see* (5).*

(b) *Results from the Finite Calculus* (1, 2, 3, 4).*

(1) *Operations.*

Whereas, if $u(x)$ be some function of x, the Differential Calculus is concerned with the properties of

$$\frac{d}{dx}\, u(x) = \lim_{h \to 0} \frac{u(x+h) - u(x)}{h}$$

the Difference Calculus deals with

$$\underset{h}{\triangle}\, u(x) = \frac{u(x+h) - u(x)}{h} \quad \text{(i)}$$

i.e., it deals with discrete quantities which can be displayed in a table; h is the length of the interval between two consecutive values of x. This quantity is called the *First Advancing Difference* of $u(x)$; the *n-th* Advancing Difference is defined as

$$\triangle^n u(x) = \triangle\, [\triangle^{n-1} u(x)].$$

If $u(x)$ is a function whose values are given for the values $x_0, x_1, \ldots, x_n$ of the variable x, the Divided Difference of $u(x)$ for the arguments x_0, x_1, is denoted by $[x_0x_1]$ and is defined by

$$[x_0x_1] = \frac{u(x_0) - u(x_1)}{x_0 - x_1}$$

* Raised figures in parentheses refer to list of References at the end of the paper.

while the divided difference of the three arguments x_0, x_1, x_2, is defined by

$$[x_0x_1x_2] = \frac{[x_0x_1] - [x_1x_2]}{x_0 - x_2}, \text{ etc.}$$

Since all our work is based on unit interval (i) becomes

$$\triangle\, u(x) = (E - 1)\, u(x)$$

where the operator E is defined as $E^r u(x) = u(x + r)$.

If the Central Difference Operator, δ, defined by

$$\delta^{2n} u(k) = \triangle^{2n} u\ (k-n)$$
$$\delta^{2n+1} u\ (k + \tfrac{1}{2}) = \triangle^{2n+1} u\ (k-n)$$

is introduced, we find

$$\begin{aligned}\delta\, u(x) &= u(x + \tfrac{1}{2}) - u(x - \tfrac{1}{2})\\ &= (E^{\frac{1}{2}} - E^{-\frac{1}{2}})\, u(x)\\ &= \triangle\ E^{-\frac{1}{2}}\, u(x).\end{aligned}$$

If two adjacent entries are *averaged*, the operation is denoted by

$$\begin{aligned}\mu\, u(x) &= \tfrac{1}{2}\, [u\ (x + \tfrac{1}{2}) + u(x - \tfrac{1}{2})]\\ &= \tfrac{1}{2}\, [E^{\frac{1}{2}} + E^{-\frac{1}{2}}]\, u(x).\end{aligned}$$

Defining the *indefinite sum* as

$$\Sigma\ u(x) = u_{x-1} + u_{x-2} + \ldots$$

it follows that $\triangle\Sigma\ u(x) = u(x)$, so that in this sense Σ is an operation inverse to $\triangle$. Therefore, the *n-th* sum is expressed by $\Sigma^n u(x) = \Sigma[\Sigma^{n-1} u(x)]$. Furthermore, the problem of finding the *definite sum*

$$\sum_a^b u(x) = u\ (a) + u\ (a + 1) + \ldots + u\ (b)$$

is evidently solved if we know a function $U(x)$ such that $\triangle U(x) = u(x)$.

Leibnitz' well known theorem in the Differential Calculus

$$D^n\ (u_x v_x) = \sum_{s=0}^{n} \binom{n}{s}\ D^{n-s}\, u(x)\ D^s v(x)$$

has an analogue in the Finite Calculus

$$\triangle^n u(x)\ v(x) = \sum_{s=0}^{n} \binom{n}{s}\ \triangle^{n-s}\, u(x + s)\ \triangle^s\, v(x) \ldots\ldots\ldots\ldots \quad \text{(ii)}$$

with, as special case

$$\triangle\ u(x)\ v(x) = u\ (x + 1)\ \triangle\ v(x) + v(x)\ \triangle\ u(x).$$

Corresponding formulae for summation can be derived :—

$$\Sigma\ u(x)\ v(x) = u(x)\ \Sigma v(x) - \Sigma[\triangle u(x)\ \Sigma\ v\ (x + 1)] \ldots\ldots\ldots \quad \text{(iii)}$$

and, in the case where $u(x)$ is a polynomial of the *n-th* degree :

$$\begin{aligned}\Sigma\ u(x)\ v(x) = u(x)\ \Sigma\ v(x) - \triangle\ u(x)\ \Sigma^2\, v(x + 1) + \triangle^2\, u(x)\ \Sigma^3\, v(x + 2)\\ - \ldots (-)^n\ \triangle^n u(x)\ \Sigma^{n+1}\, v(x + n) \ldots\ldots\ldots \quad \text{(iv)}\end{aligned}$$

2) *Factorial Notation and Properties.*

The Binomial Theorem is well-known :—

$$(a+b)^n = a^n + na^{n-1}b + \frac{n(n-1)}{1\cdot 2}a^{n-2}b^2 + \ldots + \frac{n(n-1)(n-2)\ldots(n-r+1)}{r!}$$

$$a^{n-r}b^r + \ldots + b^n$$

Now, $x^{(r)} = x(x-1)(x-2)\ldots(x-r+1)$ is defined as a *descending factorial,* and by dividing both sides by $r!$ the *reduced* descending factorial

$$x_{(r)} = \frac{x^{(r)}}{r!} = \binom{x}{r}$$

is obtained. The analogy with the binomial coefficients is clear.

It can be easily established that

$$\triangle x^{(r)} = rx^{(r-1)}$$
$$\triangle x_{(r)} = x_{(r-1)}$$

and, continuing the process, we find

$$\triangle^s x^{(r)} = r(r-1)(r-2)\ldots(r-s+1)\,x^{(r-s)}$$
$$\triangle^s x_{(r)} = x_{(r-s)} \qquad \text{(v)}$$

From the above it follows that

$$\Sigma x^{(r)} = x^{(r+1)}/_{(r+1)}$$
$$\Sigma x_{(r)} = x_{(r+1)}.$$

Describing *central factorials* as that product of factors, where the factors are in arithmetical progression of common difference unity, and centred at x, e.g.,

$$x^{[2]} = (x+\tfrac{1}{2})(x-\tfrac{1}{2})$$
$$x^{[3]} = (x+1)\,x\,(x-1)$$

we find, in general,

$$x^{[r]} = [x + \tfrac{1}{2}(r-1)]^{(r)}$$
$$= [x+\tfrac{1}{2}(r-1)][x+\tfrac{1}{2}(r-3)][x+\tfrac{1}{2}(r-5)]\ldots$$
$$[x-\tfrac{1}{2}(r-5)][x-\tfrac{1}{2}(r-3)][x-\tfrac{1}{2}(r-1)]$$

with the special cases, where the exponent is even and odd respectively

$$x^{[2s]} = (x^2-\tfrac{1}{4})(x^2-\tfrac{9}{4})\ldots(x^2-\overline{s-\tfrac{1}{2}}^2) \qquad \text{(vi)}$$
$$x^{[2s+1]} = x(x^2-1)(x^2-4)\ldots(x^2-s^2) \qquad \text{(vii)}$$

If the averaging operation μ is applied to the central factorials, we derive the *mean central factorials,* defined as

$$\mu x^{[r]} = x^{[r-1]+1}$$

with, as special cases

$$x^{[2s]+1} = x(x^2-\tfrac{1}{4})\ldots(x^2-\overline{s-\tfrac{1}{2}}^2) \qquad \text{(viii)}$$
$$x^{[2s+1]+1} = x^2(x^2-1)\ldots(x^2-s^2) \qquad \text{(ix)}$$

By dividing both classes of factorials by $r!$ the reduced cases are formed.

In exactly the same way as with the descending factorials, we find the following *properties* :

$$\delta x^{[r]} = r x^{[r-1]}$$

$$\delta^s x^{[r]} = r\,(r-1)\,(r-2)\ldots(r-s+1)\; x^{[r-s]} \ldots\ldots\ldots\ldots\ldots \quad \text{(x)}$$

$$\delta x^{]r-1[+1} = r\; x^{[r-2]+1}$$

$$\delta^s x^{[r-1]+1} = r\,(r-1)\,(r-2)\ldots(r-s+1)\; x^{[[r-s]-1]+1} \ldots\ldots \quad \text{(xi)}$$

(3) *Interpolation Formulae.*

Since it can be shown that the *n–th* difference of a polynomial of degree n is constant, the following formulae are exact if applied to a polynomial function, and consequently, the remainder term is not shown.

Newton's Divided Difference Formula :

$$u(x) = u(x_1) + \sum_{s=1}^{n-1} (x - x_1)\,(x - x_2)\ldots(x - x_s)\,[x_1 x_2 \ldots x_{s+1}] \ldots\ldots \quad \text{(xii)}$$

Gregory–Newton Formula :

$$u_x = u_0 + x\Delta u_0 + x_{(2)}\Delta^2 u_0 + x_{(3)}\Delta^3 u_0 + \ldots\ldots\ldots\ldots \quad \text{(xiii)}$$

Newton–Stirling formula (odd No. of values of $u(x)$ with central value $u(o)$:

$$u_x = u_0 + x\cdot\mu\delta u_0 + \mu x^{[2]}\delta^2 u_0/2! + x^{[3]}\cdot\mu\delta^3 u_0/3! + \ldots\ldots\ldots \quad \text{(xiv)}$$

Newton–Bessel formula (even No. of values with two central values $u\,(-\frac{1}{2})$ and $u\,(\frac{1}{2})$:

$$u_x = \mu u_0 + \mu x\cdot\delta u_0 + x^{[2]}.\mu\delta^2 u_0/2! + \mu x^{[3]}\cdot\delta^3 u_0/3! + \ldots\ldots\ldots \quad \text{(xv)}$$

(4) *Identities in Central and Mean Central Factorials.*

The elegantness of Aitken's derivation depends on certain less familiar factorial identities, of which the limiting case is $(x^2 - q^2)^r$, allowing a perfect analogy with the continuous Legendre polynomial.

We have :

(a) $x^2 - q^2 = (x^2 - \frac{1}{4}) - (q^2 - \frac{1}{4})$

(b) $(x^2 - \overline{q + \tfrac{1}{2}}^2)(x^2 - \overline{q - \tfrac{1}{2}}^2) = x^2(x^2 - 1) - 2(x^2 - 1)(q^2 - \frac{1}{4}) + (q^2 - \frac{1}{4})(q^2 - 9/4)$
$= (x^2 - \frac{1}{4})(x^2 - 9/4) - 2(x^2 - \frac{1}{4})(q^2 - 1) + q^2(q^2 - 1)$

(c) $(x^2 - \overline{q + 1}^2)(x^2 - q^2)(x^2 - \overline{q - 1}^2) = x^2(x^2 - 1)(x^2 - 4) - 3x^2(x^2 - 1)(q^2 - 1) + 3(x^2 - 1)q^2(q^2 - 1) - q^2(q^2 - 1)(q^2 - 4)$
$= (x^2 - \frac{1}{4})(x^2 - 9/4)(x^2 - 25/4) - 3(x^2 - \frac{1}{4})(x^2 - 9/4)(q^2 - 9/4) + 3(x^2 - 9/4)(q^2 - \frac{1}{4})(q^2 - 9/4) - (q^2 - \frac{1}{4})(q^2 - 9/4)(q^2 - 25/4)$

(d) $(x^2 - \overline{q + {}^3/_2}^2)(x^2 - \overline{q + \tfrac{1}{2}}^2)(x^2 - \overline{q - \tfrac{1}{2}}^2)(x^2 - \overline{q - {}^3/_2}^2) =$
$a\,b\,c\,d - 4\,a\,b\,c\,B + 6\,bc\,AB - 4\,c\,A\,B\,C + A\,B\,C\,D$
$= a'b'c'd' - 4\,a'b'c'C' + 6\,a'b'B'C' - 4b'A'B'C' + A'B'C'D'$

where $abcd$ denotes $x^2(x^2 - 1)(x^2 - 4)(x^2 - 9)$, $ABCD$ denotes $(q^2 - \frac{1}{4})(q^2 - 9/4)(q^2 - 25/4)\; q^2 - 49/4)$

and $a'b'c'd'$ denotes $(x^2-\frac{1}{4})(x^2-9/4)(x^2-25/4)(x^2-49/4)$ $\quad A'B'C'D'$ denotes $q^2(q^2-1)(q^2-4)(q^2-9)$

From the above it will be seen :—

(i) Although expansions are special cases of the Newton Dividend Difference formula, in practice a combinatorial scheme can be set up.

(ii) For an odd number of factors : $f(x^2,q^2) = -f(q^2,x^2)$ and $f(x^2-\frac{1}{4}, q^2-\frac{1}{4}) = f(q^2-\frac{1}{4}, x^2-\frac{1}{4})$.

For an even number of factors : $f(x^2-\frac{1}{4}, q^2) = f(q^2-\frac{1}{4}, x^2)$ and $f(x^2, q^2-\frac{1}{4}) = f(q^2, x^2-\frac{1}{4})$

(iii) Denoting expressions on left by $I(r)$, we have as special cases :—

$$I(2s) = R(o) + \sum_{t=0}^{s} (-)^{s-t}\binom{2s}{s-t} (q^2-s^2)(q^2-\overline{s-1}^2)\ldots(q^2-\overline{t+1}^2)\, x^{[2s+2t]} \qquad \text{(xvi)}$$

and

$$I(2s+1) = R(1) + \sum_{t=0}^{s} (-)^{s-t}\binom{2s+1}{s-t} (q^2-s^2)(q^2-\overline{s-1}^2\ldots(q^2-\overline{t+1}^2)\, x^{[2s+2t+1]} \qquad \text{(xvii)}$$

where $R(o)$ and $R(1)$ are aggregates of terms falling away with the $2s-th$ and $(2s+1)$ *th* differences respectively.

(5) *Examples of Tables of Differences.*

			u_0			
		Σu_1		Δu_0		
	$\Sigma^2 u_2$		u_1		$\Delta^2 u_0$	
$\Sigma^3 u_3$		Σu_2		Δu_1		$\Delta^3 u_0$
	$\Sigma^2 u_3$		u_2		$\Delta^2 u_1$	
$\Sigma^3 u_4$		Σu_3		Δu_2		$\Delta^3 u_1$
	$\Sigma^2 u_4$		u_3		$\Delta^2 u_2$	
$\Sigma^3 u_5$		Σu_4		Δu_3		
	$\Sigma^2 u_5$		u_4			
		Σu_5				

Thus the above elaborated for x^3 becomes for $x = 0, 1, 2, 3, 4$:

Sums.			*Function.*	*Differences.*		
			0			
		0		1		
	0		1		6	
0		1		7		6
	1		8		12	
1		9		19		6
	10		27		18	
11		36		37		
	46		64			
		100				

Central Differences: *n odd*

$$\begin{array}{lllll}
u_{-2} & & & & \\
 & \delta u_{-3/2} & & & \\
u_{-1} & & \delta^2 u_{-1} & & \\
 & \delta u_{-1/2} & & \delta^3 u_{-1/2} & \\
u_0 & & \delta^2 u_0 & & \delta^3 u_0 \\
 & \delta u_{1/2} & & \delta^3 u_{1/2} & \\
u_1 & & \delta^2 u_1 & & \\
 & \delta u_{3/2} & & & \\
u_2 & & & &
\end{array}$$

n even

$$\begin{array}{llll}
u_{-5/2} & & & \\
 & \delta u_{-2} & & \\
u_{-3/2} & & \delta^2 u_{-3/2} & \\
 & \delta u_{-1} & & \delta^3 u_{-1} \\
u_{-1/2} & & \delta^2 u_{-1/2} & \\
 & \delta u_0 & & \delta^3 u_0 \\
u_{1/2} & & \delta^2 u_{1/2} & \\
 & \delta u_1 & & \delta^3 u_1 \\
u_{3/2} & & \delta^2 u_{3/2} & \\
 & \delta u_2 & & \\
u_{5/2} & & &
\end{array}$$

3. The Approximation Problem.

Suppose we have statistical data as displayed in Table 1. By means of the Method of Least Squares we wish to fit a curve of the polynomial type, i.e.,

$$Y' = C_0 + C_1 X + C_2 X^2 + \ldots + C_r X^r \quad \ldots\ldots\ldots\ldots \quad (1)$$

Considering only the simplest case, viz., n independent observations Y_x of equal weight, corresponding to n equi-spaced values of X, the polynomial of best fit is given by the minimum of the sum of squared residuals:

$$\sum_x (Y - Y')^2 = \sum_x [Y - C_0 - C_1 X - C_2 X^2 - \ldots - C_r X^r]^2 \quad \ldots\ldots \quad (2)$$

which give $(r + 1)$ normal equations by means of which the C values are determined, expressible as

$$\sum_x X^i (Y - Y') = 0 \; (i = 0, 1, 2, \ldots, r) \quad \ldots\ldots\ldots\ldots \quad (3)$$

Table 1.

X	x	Y
a	$-\frac{1}{2}(n - 1)$	Y_0
$a + 1$	$-\frac{1}{2}(n - 3)$	Y_1
$a + 2$	$-\frac{1}{2}(n - 5)$	Y_2
.	.	.
.	.	.
.	.	.
$b - 2$	$\frac{1}{2}(n - 3)$	Y_{n-2}
$b - 1$	$\frac{1}{2}(n - 1)$	Y_{n-1}

Apart from the fact that the method becomes extremely laborious with large n and r, since the sums of powers of X up to the $2r$-*th* are required, another disadvantage attaches itself to this approach. Since we do not know in advance which degree will give a satisfactory fit, this implies that if the degree of the polynomial is changed, then the previous coefficients have to be recalculated. The advantage of using some system, which will allow the raising of the degree of the polynomial, while the coefficients already calculated retain their value, is apparent.

By transforming the power polynomial into an aggregate of special components having the property of being *uncorrelated* or *orthogonal* (Gr. *orthos*, right, *gonia*, angle) our object is attained.

If $F_r = F_r(X)$ is defined as an orthogonal polynomial of degree r, it has the properties

$$\left.\begin{array}{l} \sum\limits_a^{b-1} F_r F_s = 0 \;\; if \;\; r \neq s \ldots\ldots \\ \sum\limits_a^{b-1} F_r^2 \neq 0 \;\; if \;\; r = s \ldots\ldots \end{array}\right\} \qquad (4)$$

Y, the dependent variable, is now expressed, not in terms of *powers* of X, the determining variable, but in terms of *orthogonal polynomials* of X. Thus

$$Y' = a_0 + a_1 F_1 + a_2 F_2 + \ldots + a_r F_r$$

Utilizing properties (4), the minimizing condition (2) becomes

$$a_r \sum_a^{b-1} F_r^2 - \sum_a^{b-1} YF_r = 0$$

$$i.e., \qquad a_r = \frac{\sum\limits_a^{b-1} Y F_r}{\sum\limits_a^{b-1} F_r^2} \ldots\ldots \qquad (5)$$

Thus each coefficient a_r in the regression equation is found independently from the others, without the labour of solving simultaneous equations.

The minimum sum of squared residuals (2) becomes by (5)

$$\sum_a^{b-1} (Y - Y')^2 = \sum_a^{b-1} [Y^2 - a_0^2 - a_1^2 F_1^2 - \ldots - a_r F_r^2]$$
$$= \Sigma Y^2 - a_0 \Sigma Y - a_1 \Sigma YF_1 - \ldots - a_r \Sigma YF_r \ldots \qquad (6)$$

enabling the evaluation beforehand of what value of r will give the best polynomial Y'.

$$\text{Also } t = \frac{(a_r - a_r)\sqrt{(N - r - 1)\,\Sigma F_r^2}}{\sqrt{\Sigma\,(Y - Y')^2}}$$

will be distributed in the t-distribution with $(N - r - 1)$ degrees of freedom, and can be used to test the significance of the deviation $a_i - a_i$ of any of the coefficients from a hypothetical value a_i. In practice this hypothetical value is usually taken to be zero.

4. Derivation of $F_r(x)$.

Let $f_{r-1} = f_{r-1}(x)$ be an arbitrary polynomial of degree $(r - 1)$. Since f_{r-1} can be expressed as F_s and since $(r - l) \neq r$, we have from (4)

$$\sum_a^{b-1} f_{r-1} F_r = 0.$$

Applying formula (iv) of the Mathematical Introduction $\sum\limits_a^{b-1} f_{r-1} F_r = f_{r-1} \Sigma F_r(X) - \triangle f_{r-1} \Sigma^2 F_r(X+1) + \triangle^2 f_{r-1} \Sigma^3 F_r(X+2) - \ldots (-)^{r-1} \triangle^{r-1} f_{r-1} \Sigma^r F_r(X + r - 1) \cdot \Sigma F_r(X)$ contains an arbitrary constant which can be chosen so that $\Sigma F_r(a)$ becomes zero. In the same way $\Sigma^2 F_r(a+1)$, . . . , $\Sigma_r F_r(a + r - 1)$ vanish. In other words $[\Sigma f_{r-1} F_r]_{x=a} = O$ i.e., the definite sum vanishes for $X = a$. For $X = b$, the expression also vanishes. Since the polynomial F_{r-1} is arbitrary for all values of r, it can be deduced that $(X-a)$ and $X-b)$ are factors of the orthogonal polynomial F_r, and by successive summation it can be shown that $(X-a)_{(r)}$ and $(X-b)_{(r)}$ are multiplying factors of $\Sigma^r F_r(X)$. That is

$$\sum_a^{b-1} F_r(X) = C.\,(X-a)_{(r)}\,(X-b)_{(r)} \ldots\ldots \qquad (8)$$

where, since both sides are of degree $2r$, C is an arbitrary constant.

Taking *r-th* differences of both sides, the general expression for the orthogonal polynomials *w.r.t.* $X = a, a + 1, \ldots, b - 1$ becomes

$$F_r (X) = C. \triangle^r (X-a)_{(r)} (X-b)_{(r)} \ldots \qquad (9)$$

Applying formula (ii) it can be written

$$F_r (X) = C. \sum_{t=0}^{r} \binom{r}{t} \binom{x-a+t}{t} \binom{x-b}{r-t} \ldots \qquad (10)$$

or, utilizing formula (xiii) differenced r times with a as origin, that is

$$\triangle^r u_x = \sum_{s=0}^{n-r} (X - a)_{(s)} \triangle^{r+s} u_a$$

and expanding (8) into a Newton-series of binomial coefficients $(X - b)_{(t)}$ we have

$$\Sigma^r F_r (X) = C. \sum_{t=0}^{2r} (X - b)_{(t)} \triangle^t [(X - a)_{(r)} (X - b)_{(r)}]_{X=b}.$$

According to formula (ii)

$$\begin{aligned} \triangle^t [(X - a)_{(r)} (X - b)_{(r)}]_{X=b} &= [\sum_{s=0}^{t} t_{(s)} \triangle^{t-s} (X - a + s)_{(r)} \\ &\quad \triangle^s (X - b)_{(r)}]_{X=b} \\ &= t_{(r)} (b - a + r)_{(2r-t)} \end{aligned}$$

all other terms vanishing.

Thus

$$\Sigma^r F_r (X) = C. \sum_{t=r}^{2r} t_{(r)} (b - a + r)_{(2r-t)} (X - b)_{(t)} \ldots \qquad (11)$$

Since $t > r$, let $t = r + s$. Substituting and determining the *r-th* difference, we find

$$F_r (X) = C. \sum_{s=0}^{r} (X - b)_{(s)} (r + s)_{(r)} (b - a + r)_{(r-s)} \ldots \qquad (12)$$

Now, since $\Sigma^r F_r (X)$ is symmetric *w.r.t.* a and b, expression (12) can also be expressed in terms of the initial value of the determining variable, i.e., a. Thus, noting that $b - a = n$, and $(a - b + r)_{(r-s)} = (-)^{r-s} (n - s - 1)_{(r-s)}$ we have from (12).

$$F_r (X) = C. \sum_{s=0}^{r} (-)^{r-s} (r + s)_{(r)} (n - s - 1)_{(r-s)} (X - a)_{(s)} \ldots \qquad (12^1)$$

This is the general explicit form of the orthogonal polynomial, and the various systems proposed differ only in the value assigned to the arbitrary constant C, determined in each case by the criterion that the numerical work involved in the particular system should be a minimum.

5. Derivation of $\sum_{a}^{b-1} F_r^2 (X)$.

Applying formula (iv) to $\Sigma[F_r F_r]$, we find

$$\begin{aligned} \Sigma [F_r F_r] = F_r \Sigma F_r (X) - \triangle F_r \Sigma^2 F_r (X + 1) + \triangle^2 F_r \Sigma^3 F_r \\ (X + 2) - \ldots (-)^{s-1} \triangle^{s-1} F_r \Sigma^s F_r (X + s - 1) \\ \ldots (-)^r \triangle^r F_r \Sigma^{r+1} F_r (X + r) \ldots \qquad (13) \end{aligned}$$

If $s < r + 1$, the quantities $\Sigma^s F_r (X + s - 1)$ are easily obtained. Applying expression (11), replacing X by $X + s - 1$, it will be seen that at both limits $X = a$, $X = b$ all the terms of (11), excepting the last, vanish. Now

$$\Sigma^{r+1} F_r (X + r) = C \cdot \sum_{s=r}^{2r} (X + r - b)_{(s+1)} s_{(r)} (b - a + r)_{(2r-s)}$$

which is the expression for the indefinite sum of $\Sigma^r F_r$ vanishing for $X = b$, since $r < s + 1$.

Its value for $X = a$ will be

$$\Sigma^{r+1} F_r (a + r) = C \cdot \sum_{s=r}^{2r} s_{(r)} (a - b + r)_{(s+1)} (b - a + r)_{(2r-s)}$$

and, utilizing the fact that $b - a = n$, it becomes

$$\Sigma^{r+1} F_r (a + r) = C \cdot (n + r)_{(2r+1)} \sum_{s=r}^{2r} (-)^{s+1} s_{(r)} (2r + 1)_{(s+1)}$$

By combinatory analysis the sum on the righthand-side is equal to $(-1)^{r+1}$, giving

$$\Sigma^{r-1} F_r (a + r) = (-)^{r+1} C \cdot (n + r)_{(2r+1)}$$

From (12), $\triangle^r F_r (X) = C (2r)_{(r)}$, and, on combining these quantities into the definite sum of (13), the general expression for the sums of squares of the orthogonal polynomials is derived

$$\sum_a^{b-1} F_r^2 = C^2 \cdot (2r)_{(r)} (n + r)_{(2r+1)} \qquad (14)$$

6. Determination of $\sum_a^{b-1} YF_r (X)$.

If $Y' \doteq a_0 + a_1 F_1 + a_2 F_2 + \ldots + a_r F_r$ it was shown in section 3 that the coefficients a_r can be determined from the data, by means of the formula

$$a_r = \frac{\Sigma YF_r}{\Sigma F_r^2} \qquad (5)$$

Since the denominator is independent of the origin of X, and only depends upon the values of n, the number of data, and r, the degree of the polynomial, it only becomes necessary to evaluate the product.

It can be shown that, if the origin of X is chosen suitably, this product can be evaluated by a process of consecutive summation. Since F_r is expressed either in the form of a Gregory–Newton series (taking the initial datum as origin) or in the form of a Newton–Stirling or Newton–Bessel series (*see* Section 8), depending upon whether the number of data are odd or even (taking the origin at the centre of the data), consecutive summation will respectively yield the reduced factorial moments and reduced central factorial moments, which, when combined according to the formula in question, will yield the numerator of the expression in (5).

For practical purposes, however, if n and r are not too large, the direct multiplication method according to expression (5), with the aid of the standard tables of F_r (Appendix 1), and a calculating machine, is far superior.

For large n and r (usually not met with in practice), some summation method will probably be preferred, and the reader is referred to ([38]) and ([41]) for a succint description.

7. Compilation of the Standard Tables.

If, in the expression for $F_r (x)$, we omit the arbitrary constant C for the moment, we have

$$\begin{aligned} F_r(X) &= \sum_{s=0}^{r} (-)^{r-s} (r + s)_{(r)} (n - s - 1)_{(r-s)} (X - a)_{(s)} \\ &= (2r)_{(r)} (X - a)_{(r)} - (2r - 1)_{(r)} (n - r) (X - a)_{(r-1)} + \\ &\quad (2r - 2)_{(r)} (n - r + 1)_{(2)} (X - a)_{(r-2)} - \ldots \\ &\quad \ldots (-)^{r-1} (r + 1)_{(r)} (n - 2)_{(r-1)} (X - a) + (-)^r \\ &\quad (n - 1)_{(r)} \end{aligned} \qquad (15)$$

Differencing this expression t times, utilizing formula (v)

$$\triangle^t F_r(X) = (2r)_{(r)}(X-a)_{(r-t)} - (2r-1)_{(r)}(n-r)(X-a)_{(r-t+1)} + (2r-2)_{(r)}(n-r+1)_{(2)}(X-a)_{(r-t-2)} - \ldots$$
$$\ldots (-)^{r-4}(r+1)_{(r)}(n-2)_{(r-1)}(X-a)_{(1-t)} \ldots \quad (16)$$

Putting $X = a$, these two expressions can be combined into one formula, viz.,

$$\triangle^t F_r(a) = (-)^{r-t}(r+t)_{(r)}(n-t-1)_{(r-t)} \ldots \quad (17)$$

giving the initial difference of $F_r(x)$ for $X = a$.

As special cases we find

$$F_r(a) = (-)^r (n-1)_{(r)}.$$
$$\triangle F_r(a) = (-)^{r-1}(r+1)_{(r)}(n-2)_{(r-1)}.$$
$$\triangle^2 F_r(a) = (-)^{r-2}(r+2)_{(r)}(n-3)_{(r-2)}.$$
$$\cdot \quad \cdot \quad \cdot$$
$$\cdot \quad \cdot \quad \cdot$$
$$\cdot \quad \cdot \quad \cdot$$
$$\triangle^{r-2} F_r(a) = (2r-2)_{(r)}(n-r+1)_{(2)}.$$
$$\triangle^{r-1} F_r(a) = -(2r-1)_{(r)}(n-r).$$
$$\triangle r F_r(a) = (2r)_{(r)}.$$

yielding, e.g., for $r = 5$, $n = 7$.

$$F_5(a) = -5_{(5)}6_{(5)}.$$
$$\triangle F_5(a) = 6_{(5)}5_{(4)}.$$
$$\triangle^2 F_5(a) = -7_{(5)}4_{(3)}.$$
$$\triangle^3 F_5(a) = 8_{(5)}3_{(2)}.$$
$$\triangle^4 F_5(a) = -9_{(5)}2_{(1)}.$$
$$\triangle^5 F_5(a) = 10_{(5)}1_{(0)}.$$

Removing common factor 6, we find consecutively -1, 5, -4, 28, -42, 42 for the the leading term and differences, enabling us by summation to build up the table of values for $F_5(X)$ with $n = 7$.

It will be noticed that each number in the above scheme consists of the product of two factors, both of which can be easily derived from the *figurate number* properties (modification of the well-known Pascal triangle algorithm) of binomial coefficients in the following way: write down $r + 1$ columns of figurate numbers from right to left. Multiply the values in each column by the first $r + 1$ values in the $(r + 1)$-*th* column with alternate + and $-$ signs from the right. Remove common factors within each row; the rows in the table of products give the leading term and initial differences for values of n from $(r + 1)$ upwards.

Example: $r = 5$, $n = 6, 7, 8, \ldots$

First Step: *Figurate Numbers.*

n						
6	1	1	1	1	1	1
7	6	5	4	3	2	1
8	21	15	10	6	3	1
9	56	35	20	10	4	1
10	126	70	35	15	5	1
11	252	126	56	21	6	1
12	462	210	84	28	7	1

Each number in any column after the first from the right is the progressive sum of the numbers in the column to the right up to the row, in which it stands, e.g., 35 (in the 5-*th* column) = 1 + 4 + 10 + 20.

Second Step: Multiplication.

n	$F_r(a)$	$\Delta F_r(a)$	$\Delta^2 F_r(a)$	$\Delta^3 F_r(a)$	$\Delta^4 F_r(a)$	$\Delta^5 F_r(a)$
6	— 1	6	— 21	56	—126	252
7	— 6	30	— 84	168	—252	252
8	— 21	90	—210	336	—378	252
9	— 56	210	—420	560	—504	252
10	—126	420	—735	840	—630	252
11	—252	756	—1176	1176	—756	252
12	—462	1260	—1764	1568	—882	252

Third Step: Removing Common Factor.

n							Common Factor
6	— 1		— 21	56	—126	252	1
7	— 1	5	— 14	28	— 42	42	6
8	— 7	30	— 70	112	—126	84	3
9	— 4	15	— 30	40	— 36	18	14
10	— 6	20	— 35	40	— 30	12	21
11	— 3	9	— 14	14	— 9	3	84
12	—33	90	—126	112	— 63	18	14

Fourth Step: Derivation of $F_r(X)$ by Multiplication.

By summation from the leading term and initial differences the values of $F_r(X)$ for the different values of X can be found. In compiling our standard tables (Appendix I) it was found easier to substitute a process of multiplication, as follows:—

Since $F_r(X)$ is nothing else than a Gregory–Newton interpolation formula, it can be written

$$F_r(X) = F_r(a) + (X-a)\Delta F_r(a) + (X-a)_{(2)}\Delta^2 F_r(a) + \dots + (X-a)_{(r)}\Delta^r F_r(a) \quad (18)$$

Substituting respectively $X = a, a + 1, \ldots$ in this expression, we derive

$$F_r(a) = F_r(a)$$
$$F_r(a + 1) = F_r(a) + \triangle F_r(a)$$
$$F_r(a + 2) = F_r(a) + 2 \triangle F_r(a) + \triangle^2 F_r(a)$$
$$F_r(a + 3) = F_r(a) + 3 \triangle F_r(a) + 3 \triangle^2 F_r(a) + \triangle^3 F_r(a), \text{ etc.}$$

Denoting $\triangle^t F_r(a)$ by $(-)^{r-t} A_r$, where A_r is the absolute value of $\triangle^t F_r(a)$ we have respectively for r = *even* and r = *odd*.

X	F_{2s}	F_{2s+1}
a	A_0	$-A_0$
$a+1$	$A_0 - A_1$	$-A_0 + A_1$
$a+2$	$A_0 - 2A_1 + A_2$	$-A_0 + 2A_1 - A_2$
$a+3$	$A_0 - 3A_1 + 3A_2 - A_3$	$-A_0 + 3A_1 - 3A_2 + A_3$
$a+4$	$A_0 - 4A_1 + 6A_2 - 4A_3 + A_4$	$-A_0 + 4A_1 - 6A_2 + 4A_3 - A_4$

It is observed that the coefficients of the A's form a Pascal triangle, i.e., they correspond to the diagonals of the figurate numbers in Step 1. In this way the table of figurate numbers is used twice, viz., first to find the leading differences of an orthogonal polynomial, and then showing how to combine these differences to obtain the values for different X.

It will now be recalled that the arbitrary constant C was temporarily omitted for this discussion. Since we have used the Aitken-criterion (Section 11), viz., $C = 1$, the above development is immediately applicable. In the case of the other systems, all results above must be multiplied by the value C assumes in that particular development. In practice this amounts to multiplying the common factor removed by C.

As can be seen from the derivation in Section 4, $F_r(X)$ is symmetrical in absolute value about the centre of the data, the same signs thus occuring in the upper and lower portions of the table when r is even, opposite signs when r is odd. Thus it is only necessary to apply the derivation methods to one half of the table.

8. The Derivation of the Central Case : $\hat{F}_r(x)$.

In section 4 we derived

$$F_r(X) = C \triangle^r (X - a)_{(r)} (X - b)_{(r)} \ldots\ldots\ldots \quad (8)$$

where X took the values a to $b - 1$, or, the same thing, a to $a + n - 1$. Measure X from the centre of the data, thus $x = X - \bar{X}$, and transform to central difference notation by the relation $\triangle^r u(x) = \delta^r u(x + r/_2)$.

Using Aitken's criterion, let $C = 1$ and put $n = 2q$, thus finding

$$\hat{T}_r(x) = \delta^r [(x + q + \tfrac{1}{2}\overline{r - 1})_{(r)} (x - q + \tfrac{1}{2}\overline{r - 1})_{(r)}]$$

$$= \frac{\delta^r}{(r!)^2} [(x + q + \tfrac{1}{2}\overline{r - 1})(x + q + \tfrac{1}{2}\overline{r - 3}) \ldots (x + q - \tfrac{1}{2}\overline{r - 3})$$
$$(x + q - \tfrac{1}{2}\overline{r - 1}) \cdot (x - q + \tfrac{1}{2}\overline{r - 1})(x - q + \tfrac{1}{2}\overline{r - 3})$$
$$\ldots (x - q - \tfrac{1}{2}\overline{r - 3})(x - q - \tfrac{1}{2}\overline{r - 1})]$$

where $\hat{T}_r$ now denotes the orthogonal polynomial. By appropriate multiplication within the brackets we readily find

$$\hat{T}_r(x) = \frac{\delta^r}{(r!)^2}[(x^2 - \overline{q + \tfrac{1}{2}(r-1)}^2 (x^2 - \overline{q + \tfrac{1}{2}(r-3)}^2 \dots$$
$$(x^2 - \overline{q - \tfrac{1}{2}(r-3)}^2 (x^2 - \overline{q - \tfrac{1}{2}(r-1)}^2 \dots\dots\dots \quad (19)$$

By means of the factorial identities, Section 2, the general case can be solved, but it is more instructive to treat the even and odd cases separately.

If r is even, i.e., $r = 2s$, it follows that, using formulae (xvi) and (x)

$$\hat{T}_{2s}(x) = \frac{\delta^{2s}}{(2s)!\,(2s)!} I_{2s}$$
$$= \sum_{t=o}^{s} (-)^{s-t} (2s)_{s-t} \frac{(2s+2t)(2s+2t-1)\dots(2t+1)}{(2s)!\ \ (2s)!}$$
$$(q^2 - s^2)\dots(q^2 - \overline{t+1}^2)\, x^{[2t]}$$
$$= \sum_{t=o}^{s} (-)^{s-t} (2s+2t)_{(2t)} \frac{(q^2 - s^2)\dots(q^2 - \overline{t+1}^2)}{(s+t)!\ \ (s-t)!} x^{[2t]} \dots\dots \quad (20)$$

Using formulae (xvii) and (xi) we find

$$\hat{T}_{2s+2}(x) = \sum_{t=o}^{s} (-)^{s-t} (2s+2t+2)_{(2t+2)} \frac{(q^2-s^2)\dots(q^2-\overline{t+1}^2)}{(s+t+1)!\ \ (s-t)!} \mu x^{[2t+1]} \quad (21)$$

It will be noted that the above formulae are suitable for application to cases with an *even* number of data. By utilizing the alternative forms of the factorial identities, two corresponding expressions are derived for an odd number of data, viz.,

$$\hat{T}_{2s}(x) = \sum_{t=o}^{s} (-)^{s-t} (2s+2t)_{(2t)} \frac{(q^2-\overline{s-{}^1/_2}^2)(q^2-\overline{s-{}^3/_2}^2)\dots(q^2-\overline{t+{}^1/_2}^2)}{(s+t)!\ \ (s-t)!}$$
$$\mu x^{[2t]} \dots\dots\dots \quad (22)$$

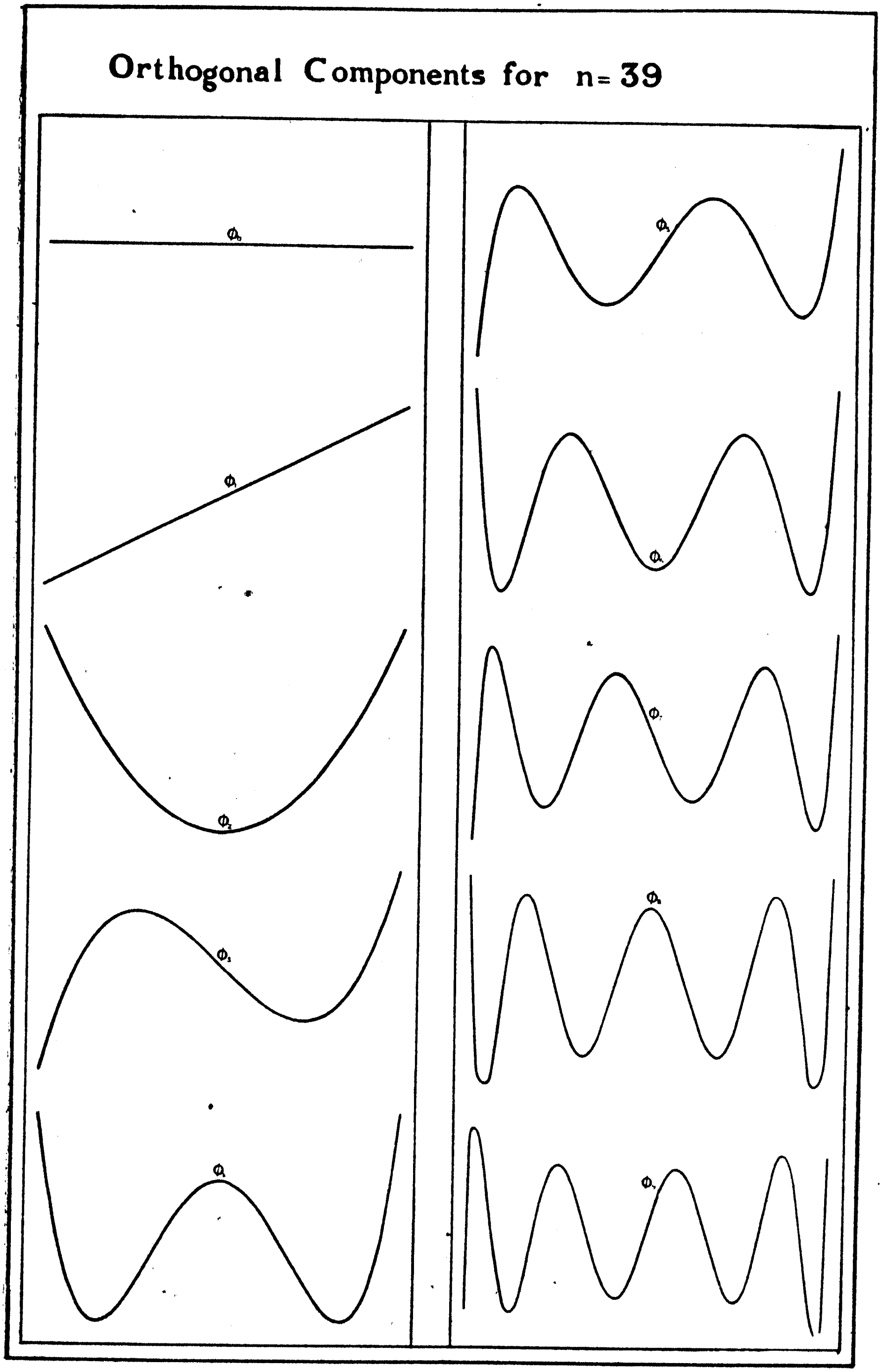
and

$$\hat{T}_{2s+1}(x) = \sum_{t=o}^{s} (-)^{s-t} (2s+2t+2)_{(2t+1)} \frac{(q^2-\overline{s+{}^1/_2}^2)(q^2-\overline{s-{}^1/_2}^2)\dots(q^2-\overline{t+{}^3/_2}^2)}{(s+t+1)!\ \ (s-t)!}$$
$$x^{[2t+1]} \dots\dots\dots \quad (23)$$

In Table 2 the explicit expressions for the two cases are given up to the ninth degree. It will be noted that the polynomials are but special cases of the Newton–Stirling (if n is odd) and the Newton-Bessel (if n is even) formulae of interpolation, thus revealing very lucidly the interpolatory character of this method of curve fitting. This fact also enables us to draw up tables, giving the central and mean central differences, from which standard tables (which are of course the same as those obtained previously) may be computed. From these central and mean central differences, the reduced factorial and reduced central factorial moments can also be obtained, and, as pointed out in Section 6, the fitting-process may then be carried out by a process of consecutive summation [38].

Figure 1, giving the graphical representation to these orthogonal polynomials for $n = 39$, is intended to bring out the extreme flexibility inherent in this method of curve fitting. Note that with increasing r, there is a proportionate increase of turning-points in the graphs.

FIGURE 1.



9. Simplified Polynomials.

Although Table 2 gives the explicit form of the orthogonal polynomials for the various degrees, a simplified form is used in practice in most applications of orthogonal polynomial fitting. This is defined as

$$\varphi_r(x) = {}^1/\lambda\ \hat{T}_r(x) \quad \ldots\ldots \quad (24)$$

where λ is the common factor explained in Section 7. The regression equation becomes

$$Y' = a'_0 + a'_1\,\varphi_1 + a'_2\,\varphi_2 + \ldots + a'_r\,\varphi_r \quad \ldots\ldots \quad (25)$$

$$a'_r = \frac{\Sigma Y\varphi_r}{\Sigma\varphi_r{}^2} = \lambda\frac{\Sigma Y\hat{T}_r}{\Sigma\hat{T}_r{}^2} = \lambda a_r \quad \ldots\ldots \quad (26)$$

Thus by removing the common factor, the normalized property falls away, although the system still remains orthogonal. Utilizing (24) and (26), the minimum sum of squared residuals becomes

$$\Sigma(Y-Y')^2 = \Sigma Y^2 - a'_0\Sigma Y - a'_1\,\Sigma Y\varphi_1 - Y'_2\Sigma Y\varphi_2 - \ldots - a'_r\,\Sigma Y\varphi_r \quad (27)$$

and the significance test

$$t = \frac{(a'_r - a_r)\sqrt{(N-r-1)\,\Sigma\varphi_r{}^2}}{\sqrt{\Sigma(Y-Y')^2}} \quad \ldots\ldots \quad (28)$$

10. Interpretation of the Constants.

Above, the practical importance of having independent constants in a regression equation, i.e., that the one could be evaluated without influencing the other, was stressed. From the significance tests it can be seen that each constant measures the importance of its approximating polynomial. This is shown in a striking way if the regression equation is written in *standard units*, i.e., multiply both sides of (25) by the reciprocal of $\sqrt{\Sigma y^2}$, taking Y from its mean, and at the same time put $\psi_r = \varphi_r/\sqrt{\Sigma\varphi_r{}^2}$.

Thus $y' = r_1\,\psi_1 + r_2\,\psi_2 + \ldots + r_r\,\psi_r \quad \ldots\ldots \quad (29)$

where $r_1, r_2, \ldots, r_r$ are the ordinary correlation coefficients between Y and the various uncorrelated polynomial values.

It thus follows that if an interpretation of the constants is being sought, the first step must be to determine the rôle that the orthogonal components play in the set of data that has to be evaluated. Since a future paper will deal with this aspect, only a few suggestions can be made here. The orthogonal components must be regarded, in a certain sense, as *standard units*, which, when multiplied by the respective constants and summated, give the best *approximation* to some, possibly unknown, functional relationship between the dependent variable and the determining variable. To take an easy, if not quite correct, analogy: Suppose we wish to describe a certain individual. We will state that Mr. Y. is 6 feet (i.e., 6 units of length) tall, weighs 170 lb. (i.e., 170 units of weight) is 30 years (i.e., 30 age-units) old, and draws a salary of £400 (i.e., 400 monetary units) per year. Since the concepts of height, weight, etc., are well-established our first task will be to establish in a corresponding way certain concepts for the orthogonal components.

Once this has been done, the manifest advantages attaching itself to the use of orthogonal polynomials in comparative studies, e.g., the evaluation of different time-series, is immediately apparent. In our example, if Mr. Y. is compared with Mr. Z., we only compare the various multiplier-constants of the standard units of height, etc.

However, a word of caution must be given when interpretations are being sought. It has been stressed throughout that the orthogonal polynomial method of curve fitting is an interpolatory, i.e., approximative, one. Thus, it is the best representation of the dependent variable in terms of *integral* values, or combinations of values, of the determining variable.

The validity of the process will depend on how far that underlying, in most cases unknown, functional relationship as influenced by " random error ", *can* be approximated by a polynomial or combination of polynomials. In other words, are our criteria, by which the set of independent polynomials were derived adequate? Research on this point seems necessary. It appears, however, that if the data conform to such conditions as to give validity to the use of the arithmetic mean, then the use of the higher constants are also permissible. In this case, the constants can be regarded either as parameters, with which the symmetry of the data can be investigated, or, as certain measures of the rates of increase. In other words, in certain cases an analogy can be set up between the parameters characterizing the one-dimensional frequency-distribution and the parameters, by means of which a two-dimensional distribution, not necessarily of a frequency-nature, can be evaluated.

Figure 2, which is a special adaptation of Figure 1, emphasizes the transformatory character of the various orthogonal components. It must be kept in mind that this transformation is of an *integral* nature, i.e., all the exponents of the determining variable are integers. That is, the straight line, parabola and third degree polynomial which are so often met with in biological curve fitting are all special cases of the procedure evaluated in the preceding pages.

11. Historical Notes.

(*a*) *Continuous Case.*

The orthogonal polynomials treated above are all discrete cases of the well-known *Legendre-polynomials*, particulars of which can be found in any standard textbook on Analysis.

If the limiting case of (19) is taken between the limits $-q$, q, Rodrigues' formula is derived

$$P_r\,(x) = \left(\frac{d}{dx}\right)^r \frac{(x^2 - q^2)^r}{(r\,!)^2}$$

The explicit values of the first few polynomials become :—

$$P_0 = 1$$

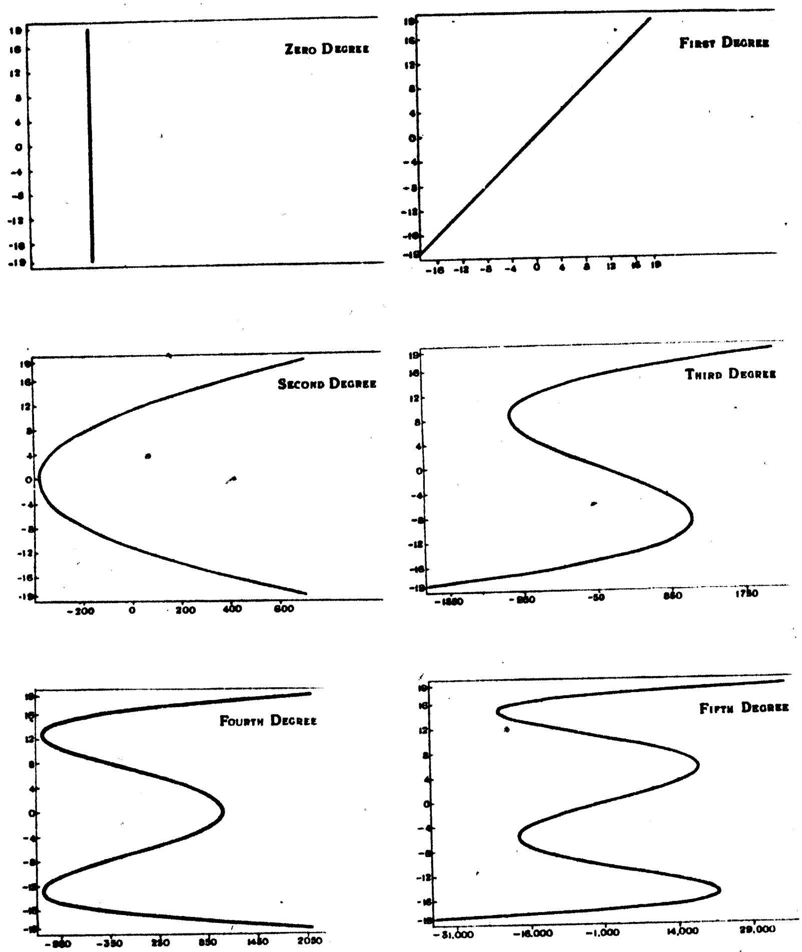
$$P_1 = 2x$$

$$P_2 = \binom{4}{2}\frac{x^2}{2!} - q^2$$

$$P_3 = \binom{6}{3}\frac{x^3}{3!} - \binom{4}{1}\frac{q^2x}{2!}$$

$$P_4 = \binom{8}{4}\frac{x^4}{4!} - \binom{6}{2}\frac{q^2x^2}{3!} + \frac{q^4}{2!\,2!}$$

FIGURE 2.



The orthogonal polynomials as transformation functions. The ordinate represents the equi-spaced values of the determining variable, while the abscissa represents the values of the orthogonal components.

(b) P. L. Chebyshev.

The problem of interpolation by means of orthogonal functions was first introduced by the famous Russian mathematician, P. L. Chebyshev, in a series of papers on orthogonal representation ([8, 9, 10, 11, 12, 13]). His researches are of a very general nature, since he treats the non-equidistant case ([8, 9]), as well as the equidistant case ([10, 11, 12, 13]) but the application in practice is extremely complicated. Using his reduction formula([11]).

$$\psi_r = 2\,(2r - 1)\, x\, \psi_{r-1} - (r - 1)^2\, [n^2 - (r - 1)^2]\, \psi_{r-2}$$

or, putting $C = (r!)^2$ and $a = -\frac{n-1}{2}$ in our (10′) the first five explicit values are

$$\psi_0 = 1$$
$$\psi_1 = 2x$$
$$\psi_2 = 12x^2 + (n^2 - 1)$$
$$\psi_3 = 120\, x^3 - 6\,(3n^2 - 7)\, x$$
$$\psi_4 = 1680\, x^4 - 120\,(3n^2 - 13)\, x^2 + 9\,(n^2 - 1)\,(n^2 - 9)$$

(c) Intermediate Period.

In his "Calcul des Probabilités"([14]), Poincaré, independently from Chebyshev, develops the interpolatory function by means of a continuous function identity for non-equidistant values. These functions are proportional to those of Chebyshev. A. Quiquet ([15]) applies this development to practical cases. J. P. Gram([17]) suggests in a general way a step-wise derivation of the general approximating function as a convergent sequence of polynomials for various orthogonal functions and applies it to the smoothing of empirical curves.

(d) Charl Jordan.

Although the theoretical basis of the orthogonal polynomials of least squares was given by Chebyshev as early as 1855, it was not until 1920, with the publication of Jordan's methods, that this approach became really practicable. In his first paper([18]) Jordan treats the mathematical theory of orthogonal polynomials for equidistant values in the general case. C was chosen as $r!/2^r\, h^r$, where h is the length of the interval, and the coefficients were derived by multiplication of the Y-values with standard values, which were calculated for the different degrees. In ([19]) the coefficients were obtained by the product of the binomial moments, obtained by successive summation, and certain standard numbers; in ([20]) mean orthogonal moments are introduced, which eliminates the calculation of ΣU_r^2, where U_r denotes the Jordan-polynomial. Our derivation of F_r is based to a certain extent upon ([22]), which paper is the final presentation of the Jordan system. Practical applications of his work will be found in ([23, 24]).

(e) Fredrik Esscher.

Denoting the orthogonal polynomial by P_r, taking X from the centre of the data, Esscher determines the value of C by the convention([25]) that the coefficient of x^r shall be unity, i.e., in our notation $C = r!/\binom{2r}{r}$. In his second paper ([26]) using polynomials $X_r(x)$, x taking the values 1, 2, 3, . . . , n, he chooses C in such a way that ΣX_r^2 becomes equal to n, i.e., in our notation

$$C = \sqrt{n \Big/ \binom{2r}{r}\binom{n+r}{2r+1}}$$

thus simplifying the expression ΣX_r^2, but complicating the polynomials.

(*f*) *P. Lorenz.*

Using the determinantal approach, Lorenz ([27], [28]) derives orthogonal polynomials $X_r(x)$, distinguishing between even and odd n, so that $\Sigma X_r^2 = n$. Thus, in our notation the value of C is found to be

$$C = \sqrt{\frac{n}{\binom{2r}{r}\binom{n+r}{2r+1}}} \quad \text{in the even case, and}$$

$$C = \frac{1}{2^{2r}} \sqrt{\frac{n}{\binom{2r}{r}\binom{n+r}{2r+1}}} \quad \text{in the odd case.}$$

For an interesting application of the Lorentz-system *see* ([30]).

(*g*) *R. A. Fisher.*

Independently from Esscher, Fisher derived his system approximately at the same time ([31] [32]). He derives his polynomials T_r, where X is measured from the mean value, so that $\Sigma T_r^2 = 1$, i.e., if the arbitrary constant in (10′) is chosen equal to $1/\sqrt{\binom{2r}{r}\binom{n+r}{2r+1}}$ and a is put equal to $-\frac{1}{2}(n-1)$, we have, e.g., for the first four explicit values

$$T_0 = \frac{1}{\sqrt{n}}$$

$$T_1 = \sqrt{\frac{12}{n(n^2-1)}}\; x$$

$$T_2 = \sqrt{\frac{180}{n(n^2-1)(n^2-4)}}\; [x^2 - \tfrac{1}{12}(n^2-1)]$$

$$T_3 = \sqrt{\frac{2800}{n(n^2-1)(n^2-4)(n^2-9)}}\; [x^3 - \tfrac{1}{20}\, x\,(3n^2-7)]$$

Later ([33]) by utilizing the convention that the coefficient of x^r shall be unity, i.e., in our notation C becoming equal to $r!/\binom{2r}{r}$, he presents his polynomials in a new form, the general expression of which is derived by Allan ([36]) as

$$\xi r = \frac{r!}{(r-\frac{1}{2})!}\left[\frac{n}{2}\right]^r \xi_1 \sum_{q=o} \frac{(-)^q\,(r-q-\frac{1}{2})!}{(r-2q)!\; q!\; 2^{2q}}\; \frac{[\xi_1]^{r-2q-1}}{[\frac{n}{2}]^{r-2q}}$$

where $[x]^n$ corresponds to $x^{[n]}$ in our notation, and where the series terminate in $\frac{1}{2}(r+1)$ or $\frac{1}{2}(r+2)$ terms. The first few polynomials become

$$\xi_0 = 1$$

$$\xi_1 = x$$

$$\xi_2 = \xi_1^2 - {}^1/_{12}\,(n^2-1)$$

$$\xi_3 = \xi_1^3 - {}^1/_{20}\,(3n^2-7)\,\xi_1$$

$$\xi_4 = \xi_1^4 - {}^1/_{14}\,(3n^2-13)\,\xi_1^2 + {}^3/_{560}\,(n^2-1)(n^2-9)$$

and $$\Sigma \xi_r^2 = \frac{(r!)^4}{(2r)!\,(2r+1)!}\, n(n^2-1)(n^2-4)\ldots(n^2-r^2)$$

In ([35]) standard tables of the polynomial values for n, from 4 to 52, and r, from 1 to 5, with common factors λ removed, are presented, while in ([34]) this method is elucidated. Thus $\xi'_r(x) = \frac{1}{\lambda}\,\xi_r(x)$. The application of the last procedure is well illustrated in ([37]).

(h) A. C. Aitken.

With the appearance of the work of Aitken ([38], [39], [40], [41]), nearly a century's search after suitable practical methods can be said to have been completed. By deriving new forms for the orthogonal polynomials in terms of central and mean central factorials the relationship between the theory of interpolation and the theory of orthogonal polynomial curve fitting is shown in a remarkably lucid way. The choice of C equal to 1 allows the double utilization of his tables of the central and mean central, and terminal values and differences, and gives integers throughout the standard tables.

Practical Example.

To illustrate the method of curve fitting, enunciated in the previous sections, we choose the average diurnal variation of atmospheric temperature during December, 1940, at Armoedsvlakte, Bechuanaland. Our aim is to express the temperature (Y) in terms of a linear combination of orthogonal polynomials (φ_r) of the time of the day (X, or x if measured from the midpoint of the data). This operation will yield—

(i) the regression equation between Y and X.

(ii) the smoothed values of the atmospheric temperature;

(iii) statistics, independently summarizing certain aspects of the diurnal variation of air temperature.

Table 3.

Example of Curve Fitting: Air Temperature.

Hour.	X.	x.	Observation.	Combinations.		φ_1.	φ_3.	φ_5.	Check. $\varphi_1+\varphi_3+\varphi_5$.
6 a.m.	1	−11·5	65·25	1−24	− 0·63	− 23	−1771	−4807	−6601
7	2	−10·5	69·37	2−23	2·66	− 21	− 847	1463	595
8	3	− 9·5	74·44	3−22	7·35	− 19	− 133	3743	3591
9	4	− 8·5	79·00	4−21	10·88	− 17	391	3553	3927
10	5	− 7·5	82·72	5−20	13·64	− 15	745	2071	2801
11	6	− 6·5	85·97	7−19	15·40	− 13	949	169	1105
12	7	− 5·5	88·11	7−18	16·21	− 11	1023	−1551	− 539
1	8	− 4·5	89·67	8−17	16·54	− 9	987	−2721	−1743
2	9	− 3·5	90·36	9−16	16·29	− 7	861	−3171	−2317
3	10	− 2·5	90·35	10−15	14·20	− 5	665	−2893	−2233
4	11	− 1·5	88·92	11−14	8·51	− 3	419	−2005	−1589
5	12	− 0·5	87·81	12−13	2·86	− 1	143	− 715	− 573
6	13	0·5	84·95	Total.	123·91	φ_2	φ_4	φ_6	$\varphi_2+\varphi_4+\varphi_6$
7	14	1·5	80·41	1+24	131·13	253	253	4807	5313
8	15	2·5	76·15	2+23	136·08	187	33	−3971	−3751
9	16	3·5	74·07	3+22	141·53	127	− 97	−4769	−4739
10	17	4·5	73·13	4+21	147·12	73	− 157	−2147	−2231
11	18	5·5	71·90	5+20	151·80	25	− 165	1045	905
12	19	6·5	70·57	7+19	156·54	− 17	− 137	3271	3117
1 a.m.	20	7·5	69·08	7+18	160·01	− 53	− 87	3957	3817
2	21	8·5	68·12	8+17	162·80	− 83	− 27	3183	3073
3	22	9·5	67·09	9+16	164·43	− 107	33	1419	1345
4	23	10·5	66·71	10+15	166·50	− 125	85	− 695	− 735
5	24	11·5	65·88	11+14	169·33	− 137	123	−2525	−2539
				12+13	172·76	− 143	143	−3575	−3575
		Total.	1,860·03	Total.	1,860·03				

Step 1.—Combine the observations as shown in column 5, Table 3. These can be checked by the relation—

Sums + Differences = 2 (Sum of first half of original observations), i.e., 123·91 + 1,860·03 = 2 (919·97).

Step 2.—Multiply these sums and differences respectively by the values of the even and odd degrees of φ_r, taken from the standard tables (Appendix I), and enter the sums of products in Table 4. The sums of products can be checked by the relations

$$\Sigma Y\varphi_1 + \Sigma Y\varphi_3 + \Sigma Y\varphi_5 = \Sigma Y\ (\varphi_1 + \varphi_3 + \varphi_5)$$

and $$\Sigma Y\varphi_2 + \Sigma Y\varphi_4 + \Sigma Y\varphi_6 = \Sigma Y\ (\varphi_2 + \varphi_4 + \varphi_6)$$

TABLE 4.

Evaluation of the Constants.

s	$\Sigma Y\ \varphi_r$	$\Sigma\ \varphi_r^2$	a_r'	Sums of Squares.
0	1,860·03	24	77·501250	144,154·650
1	− 1,311·37	4,600	− 0·285080	373·846
2	− 19,831·59	394,680	− 0·050247	996·483
3	87,266·81	17,760,600	0·004914	428·786
4	1,141·79	394,680	0·002893	3·303
5	− 78,051·97	177,928,920	− 0·000439	34·239
6	− 6,396·25	250,925,400	− 0·000003	0·163

Step 3.—Divide the sums of products $\Sigma Y\varphi_r$ by $\Sigma\varphi_r^2$, taken from standard tables, giving the a'_r values, column 4. Divide the square of $\Sigma Y\varphi_r$ by $\Sigma\varphi_r^2$, giving column 5, the sums of squares due to each term fitted.

TABLE 5.

Analysis of Variance.

Variance due to.	D.F.	S.S.	M.S.	F.	5 Per Cent.	1 Per Cent.
Total	23	1,849·395	—	—	—	—
Degree 1	1	373·846	373·846	5·57	4·30	7·94
Residual 1	22	1,475·549	67·070	S.	—	—
Degree 2	1	996·483	996·483	43·68	4·32	8·02
Residual 2	21	479·066	22·813	S.S.	—	—
Degree 3	1	428·786	428·786	170·56	4·35	8·10
Residual 3	20	50·280	2·514	S.S.	—	—
Degree 4	1	3·303	3·303	1·34	4·38	8·18
Residual 4	19	46·977	2·473	—	—	—
Degree 5	1	34·239	34·239	48·38	4·41	8·28
Residual 5	18	12·738	0·708	S.S.	—	—
Degree 6	1	0·163	0·163	0·22	4·45	8·40
Residual 6	17	12·575	0·740	—	—	—

Step 4.—From the sums of squares in Table 4 it appears that a 5-*th* degree polynomial will give a satisfactory fit, and thus, before calculating any further coefficients, a significance test by means of the analysis of variance is applied, so as to establish whether the 5-*th* degree is appropriate. This procedure is set out in Table 5. Squaring and summing the original values and subtracting the first entry in column 5, Table 4, the total S.S. value in Table 5, 1,849·395, with $(N - 1) = 23$ degrees of freedom is obtained. Subtraction of the S.S. value for the first degree from this value yields the first residual. For the second residual subtract the S.S. for the second degree from the first residual, and so on. The mean square (M.S.) column is obtained by dividing the S.S. column by the D.F. column, and the F-column is the result of dividing the M.S. for residual into the M.S. for degree. These F-values are compared with the theoretical values ([35]) in order to test significance. *S* denotes significance at 5 per cent. theoretical, while S.S. denotes significance at the 1 per cent. level. In this way significance is established for the 1st, 2nd, 3rd and 5th degrees, while the 4th degree does not attain the significance level.

This can be seen at a glance when expression (29) is utilized, giving

$$y^1 = -0{\cdot}450\ \psi_1 - 0{\cdot}734\ \psi_2 + 0{\cdot}482\psi_3 + 0{\cdot}042\ \psi_4 - 0{\cdot}136\ \psi_5.$$

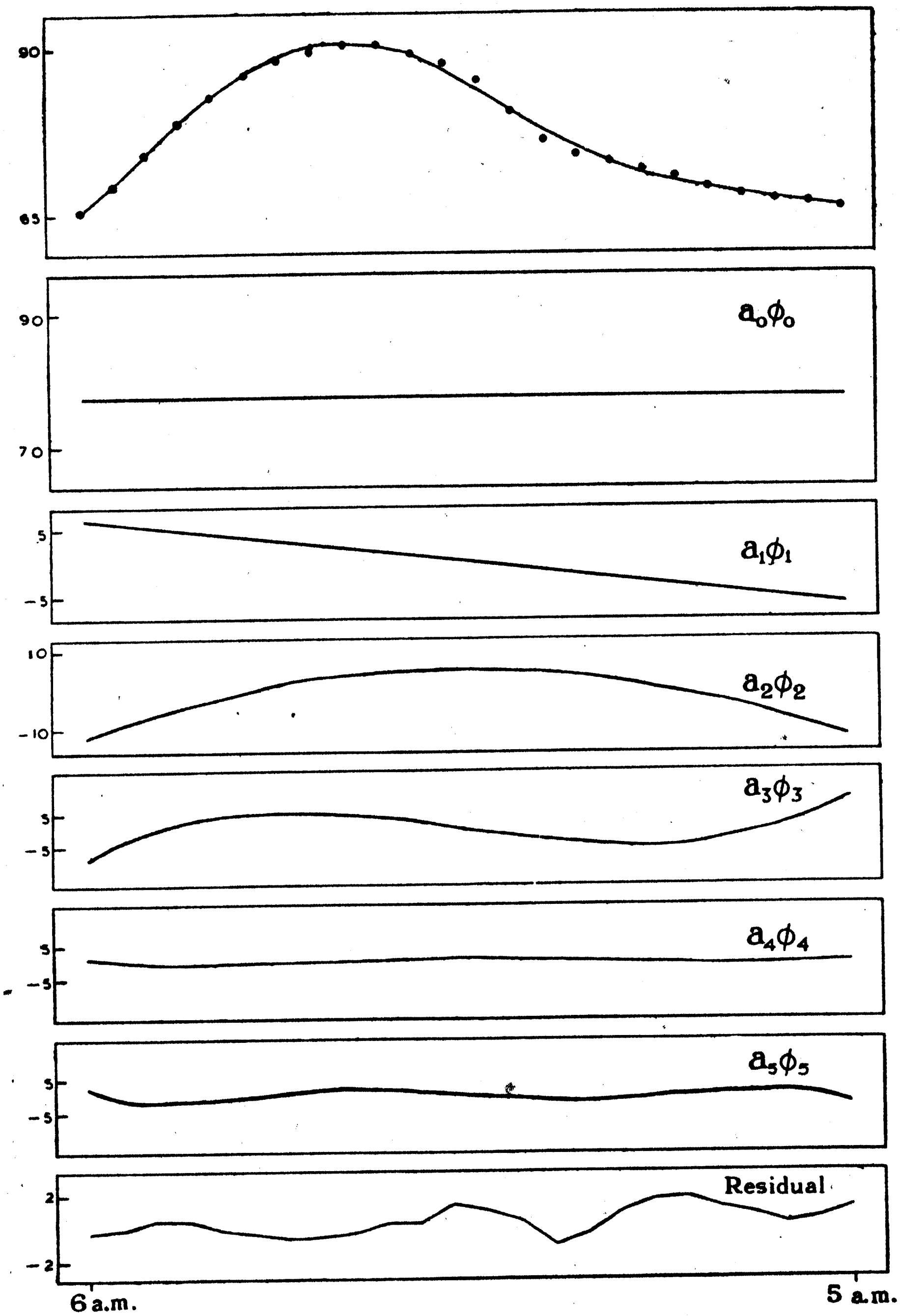
TABLE 6.

Graduated Values.

Hour.	X.	x.	$\varphi_0 a'_0$.	$\varphi_1 a'_1$.	$\varphi_2 a'_2$.	$\varphi_3 a'_3$.	$\varphi_4 a'_4$.	$\varphi_5 a'_5$.	Total.	Diff.
6 a.m.	1	−11·5	77·501	6·557	−12·712	−8·703	0·732	2·110	65·49	−0·24
7	2	−10·5	—	5·987	− 9·396	−4·162	0·0[illegible]	−0·642	69·38	−0·01
8	3	− 9·5	—	5·417	− 6·381	−0·654	−0·281	−1·643	73·96	0·48
9	4	− 8·5	—	4·846	− 3·668	1·921	−0·454	−1·560	78·59	0·41
10	5	− 7·5	—	4·276	−1·256	3·661	−0·477	−0·909	82·80	−0·08
11	6	− 6·5	—	3·706	0·854	4·663	−0·396	−0·074	86·25	−0·28
12	7	− 5·5	—	3·136	2·663	5·027	−0·252	0·681	88·76	−0·65
1	8	− 4·5	—	2·566	4·171	4·850	−0·078	1·195	90·21	−0·54
2	9	− 3·5	—	1·996	5·376	4·231	0·095	1·392	90·60	−0·23
3	10	− 2·5	—	1·425	6·281	3·268	0·246	1·270	89·99	0·36
4	11	− 1·5	—	0·855	6·884	2·059	0·356	0·880	88·54	0·38
5	12	− 0·5	—	0·285	7·185	0·703	0·414	0·314	86·40	1·41
6	13	0·5	—	—	—	—	—	—	83·80	1·15
7	14	1·5	—	—	—	—	—	—	80·95	−0·54
8	15	2·5	—	—	—	—	—	—	78·06	−1·91
9	16	3·5	—	—	—	—	—	—	75·34	−1·28
10	17	4·5	—	—	—	—	—	—	72·98	0·15
11	18	5·5	—	—	—	—	—	—	71·07	0·83
12	19	6·5	—	—	—	—	—	—	69·66	0·91
1 a.m.	20	7·5	—	—	—	—	—	—	68·75	0·34
2	21	8·5	—	—	—	—	—	—	68·17	−0·05
3	22	9·5	—	—	—	—	—	—	67·71	−0·62
4	23	10·5	—	—	—	—	—	—	67·01	−0·31
5	24	11·5	—	—	—	—	—	—	65·56	0·32
			TOTAL.	0·00	0·00	0·00	0·00	0·00	1,860·03	0·00

Step 5.—Utilizing the standard tables again, calculate $a'_0\ \varphi_0$, $a'_1\ \varphi_1$, . . . , $a'_5\,\varphi_5$ as shown in Table 6. Adding columns 4 to 9, the graduated values of Y are obtained. Because of symmetry, only the first half of the products is calculated. The graduated values of the second half is obtained by simply changing the sign of the odd degrees, leaving the signs of the even degrees unaltered, and adding. The total of the graduated

FIGURE 3.



Diurnal Variation of Air Temperature, December 1940 at Armoedsvlakte, analyzed into its orthogonal components. The circles represent the actual observations, while the smooth curve results from the addition of the components.

values should be equal to the total of the original observations. Column 11 gives the differences between the original and graduated values, the sums of squares of which should be equal to the S.S. of the 5–*th* residual in Table 5. Figure 3 shows the original values with the 5–*th* degree curve, as well as the different components.

Step 6.—The regression equation can now be written

$$Y' = 77 \cdot 501 - 0 \cdot 2851\, \varphi_1 - 0 \cdot 0502\, \varphi_2 + 0 \cdot 0049\, \varphi_3 + 0 \cdot 0029\, \varphi_4 - 0 \cdot 0004\, \varphi_5$$

or, utilizing (24) and (26), i.e., dividing each term by the common factor of the standard tables, it can be written in terms of Aitken's polynomials.

$$Y' = 77 \cdot 501 - 0 \cdot 2851\, \hat{T}_1 - 0 \cdot 0502\, \hat{T}_2 + 0 \cdot 0049\, \hat{T}_3 + 0 \cdot 00014\, \hat{T}_4 - 0 \cdot 00008\, \hat{T}_5$$

Using Table 2 and substituting for $\hat{T}_r$, we find

$$Y' = 85 \cdot 145 - 2 \cdot 613\, x - 0 \cdot 180\, x^2 + 0 \cdot 037\, x^3 + 0 \cdot 00024\, x^4 - 0 \cdot 00013\, x^5$$

and, remembering that $x = X - \bar{X}$, i.e., $x = X - 12 \cdot 5$, we finally have

$$Y' = 63 \cdot 062 + 1 \cdot 370\, X + 1 \cdot 223\, X^2 - 0 \cdot 181\, X^3 + 0 \cdot 0085\, X^4 - 0 \cdot 00013\, X^5$$

giving the relationship between air temperature and time as an ordinary power polynomial of the 5–*th* degree.

Remarks.—From this application it follows that the use of this method not only yields the graduated values, but at the same time, certain statistics, the coefficients, each of which represents some aspect of the temperature distribution in time. For instance, a'_0 is the average temperature, a'_1 is a measure of the average linear rate of increase, a'_2 is a function of the parabolic rate of increase, etc., or, a'_1 is the regression coefficient between air temperature and a certain combination of first degree time values, etc. In a forthcoming article this interpretational aspect will be fully treated.

13. Summary.

The systems of orthogonal polynomials mainly used in practice are derived from a common general formula, which is established by the principle of least squares, utilizing results from the Finite Calculus. A simplified method of utilizing the Aitken–Chebyshev polynomials, by means of an extensive set of appended standard tables, is presented.

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Appendix I.

Standard Tables.

In the following pages the values of the orthogonal polynomials are given for n, from 5 to 52, and for r, from 1 to 9. Because φ_r is symmetrical in absolute value about the centre of the date, the same signs occurring in the upper and lower portions of the table when r is even, opposite signs when r is odd, *only the first half* of the tables is given. In the first row at the base of the tables the sums of squares are given. Since these figures tend to become very large with large n and r, they have been abbreviated to 10 significant figures, e.g., for $n = 42$, $r = 8$, the sums of squares is 563, 270, 101, 173, 780; this is abbreviated to 10 figures and is denoted as 563, 270, 101, 2 (5), the 5 between

brackets denoting that 5 figures have been omitted. In the second row the common factor eliminated, is given. Below follows an example of the standard tables. For completeness sake, all the values are given in this example:—

$n = 11.$

X	x	φ_1	φ_2	φ_3	φ_4	φ_5	φ_6	φ_7
1	−5	−5	15	−30	6	−3	15	−5
2	−4	−4	6	6	−6	6	−48	23
3	−3	−3	−1	22	−6	1	29	−33
4	−2	−2	−6	23	−1	−4	36	2
5	−1	−1	−9	14	4	−4	−12	28
6	0	0	−10	0	6	0	−40	0
7	1	1	−9	−14	4	4	−12	−28
8	2	2	−6	−23	−1	4	36	−2
9	3	3	−1	−22	−6	−1	29	33
10	4	4	6	−6	−6	−6	−48	−23
11	5	5	15	30	6	3	15	5
	$\Sigma \varphi_r^2$	110	858	4,290	286	156	11,220	4,862
	λ	2	3	4	35	84	14	24

5			6				7				
φ_1	φ_2	φ_3	φ_1	φ_2	φ_3	φ_4	φ_1	φ_2	φ_3	φ_4	φ_5
−2	2	−1	−5	5	−5	1	−3	5	−1	3	−1
−1	−1	2	−3	−1	7	−3	−2	0	1	−7	4
0	−2	0	−1	−4	4	2	−1	−3	1	1	−5
							0	−4	0	6	0
10	14	10	70	84	180	28	28	84	6	154	84
2	3	4	1	2	2	5	2	3	20	5	6

8						9						
φ_1	φ_2	φ_3	φ_4	φ_5	φ_6	φ_1	φ_2	φ_3	φ_4	φ_5	φ_6	φ_7
−7	7	−7	7	−7	1	−4	28	−14	14	−4	4	−1
−5	1	5	−13	23	−5	−3	7	7	−21	11	−17	6
−3	−3	7	−3	−17	9	−2	−8	13	−11	−4	22	−14
−1	−5	3	9	−15	−5	−1	−17	9	9	−9	1	14
						0	−20	0	18	0	−20	0
168	168	264	616	2,184	264	60	2,772	990	2,002	468	1,980	858
1	3	5	5	3	7	2	1	4	5	14	7	8

10

φ_1	φ_2	φ_3	φ_4	φ_5	φ_6	φ_7
−9	6	−42	18	−6	3	−9
−7	2	14	−22	14	−11	47
−5	−1	35	−17	−1	10	−86
−3	−3	31	3	−11	6	42
−1	−4	12	18	−6	−8	56
330	132	8,580	2,860	780	660	29,172
1	6	2	7	21	28	4

11

φ_1	φ_2	φ_3	φ_4	φ_5	φ_6	φ_7
−5	15	−30	6	−3	15	−5
−4	6	6	−6	6	−48	23
−3	−1	22	−6	1	29	−33
−2	−6	23	−1	−4	36	2
−1	−9	14	4	−4	−12	28
0	−10	0	6	0	−40	0
110	858	4,290	286	156	11,220	4,862
2	3	4	35	84	14	24

12

φ_1	φ_2	φ_3	φ_4	φ_5	φ_6	φ_7	φ_8
−11	55	−33	33	−33	11	−55	11
−9	25	3	−27	57	−31	225	−61
−7	1	21	−33	21	11	−251	119
−5	−17	25	−13	−29	25	−83	−65
−3	−29	19	12	−44	4	204	−74
−1	−35	7	28	−20	−20	140	70
572	12,012	5,148	8,008	15,912	4,488	369,512	65,208
1	1	5	10	14	42	6	15

13

φ_1	φ_2	φ_3	φ_4	φ_5	φ_6	φ_7	φ_8
−6	22	−11	99	−22	22	−33	11
−5	11	0	−66	33	−55	121	−55
−4	2	6	−96	18	8	−103	89
−3	−5	8	−54	−11	43	−75	−19
−2	−10	7	11	−26	22	65	−71
−1	−13	4	64	−20	−20	100	10
0	−14	0	84	0	−40	0	70
182	2,002	572	68,068	6,188	14,212	92,378	38,038
2	3	20	5	36	42	24	45

14

φ_1	φ_2	φ_3	φ_4	φ_5	φ_6	φ_7	φ_8	φ_9
-13	13	-143	143	-143	143	-143	13	-13
-11	7	-11	-77	187	-319	473	-59	77
-9	2	66	-132	132	-11	-297	79	-163
-7	-2	98	-92	-28	227	-353	7	107
-5	-5	95	-13	-139	185	95	-65	89
-3	-7	67	63	-145	-25	375	-25	-105
-1	-8	24	108	-60	-200	200	50	-90
910	728	97,240	136,136	235,144	497,420	1,293,292	34,580	142,324
1	6	2	5	9	12	12	99	55

15

φ_1	φ_2	φ_3	φ_4	φ_5	φ_6	φ_7	φ_8	φ_9
-7	91	-91	1,001	-1,001	143	-13	91	-91
-6	52	-13	-429	1,144	-286	39	-377	494
-5	19	35	-869	979	-55	-17	415	-901
-4	-8	58	-704	44	176	-31	157	344
-3	-29	61	-249	-751	197	-3	-311	659
-2	-44	49	251	-1,000	50	25	-275	-250
-1	-53	27	621	-675	-125	25	125	-675
0	-56	0	756	0	-200	0	350	0
280	37,128	39,780	6,466,460	10,581,480	426,360	8,398	1,193,010	4,269,720
2	1	4	1	2	21	264	33	22

16

φ_1	φ_2	φ_3	φ_4	φ_5	φ_6	φ_7	φ_8	φ_9
-15	35	-455	273	-143	65	-195	65	-91
-13	21	-91	-91	143	-117	533	-247	455
-11	9	143	-221	143	-39	-143	221	-715
-9	-1	267	-201	33	59	-423	149	95
-7	-9	301	-101	-77	87	-157	-133	575
-5	-15	265	23	-131	45	235	-205	53
-3	-19	179	129	-115	-25	375	-25	-505
-1	-21	63	189	-45	-75	175	175	-315
1,360	5,712	1,007,760	470,288	201,552	77,520	1,545,232	454,480	2,846,480
1	3	1	5	21	77	33	99	55

17

φ_1	φ_2	φ_3	φ_4	φ_5	φ_6	φ_7	φ_8	φ_9
− 8	40	− 28	52	− 104	104	− 130	26	− 8
− 7	25	− 7	− 13	91	− 169	325	− 91	37
− 6	12	7	− 39	104	− 78	− 39	65	− 50
− 5	1	15	− 39	39	65	− 247	65	− 5
− 4	− 8	18	− 24	− 36	128	− 149	− 25	40
− 3	−15	17	− 3	83	93	75	− 73	19
− 2	−20	13	17	− 88	2	215	− 37	− 26
− 1	−23	7	31	− 55	− 85	175	35	− 35
0	−24	0	36	0	− 120	0	70	0
408	7,752	3,876	16,796	100,776	178,296	579,462	56,810	15,640
2	3	20	35	42	77	88	495	1,430

18

φ_1	φ_2	φ_3	φ_4	φ_5	φ_6	φ_7	φ_8	φ_9
−17	68	− 68	68	− 884	442	− 442	34	− 34
−15	44	− 20	− 12	676	− 650	1,014	− 110	146
−13	23	13	− 47	871	− 377	13	61	− 169
−11	5	33	− 51	429	169	− 715	85	− 55
− 9	−10	42	− 36	− 156	481	− 585	− 5	125
− 7	−22	42	− 12	− 588	439	31	− 77	107
− 5	−31	35	13	− 733	145	563	− 65	− 43
− 3	−37	23	33	− 583	− 209	651	7	− 133
− 1	−40	8	44	− 22[illegible]	− 440	280	70	− 70
1,938	23,256	23,256	28,424	6,953,544	2,941,884	5,794,620	78,660	211,140
1	2	10	35	7	28	44	715	715

19

φ_1	φ_2	φ_3	φ_4	φ_5	φ_6	φ_7	φ_8	φ_9
− 9	51	− 204	612	− 102	1,326	− 306	34	− 34
− 8	34	− 68	− 68	68	− 1,768	646	− 102	136
− 7	19	28	− 388	98	− 1,222	86	42	− 134
− 6	6	89	− 453	58	234	− 411	81	− 74
− 5	− 5	120	− 354	− 3	1,235	− 425	15	85
− 4	−14	126	− 168	− 54	1,352	− 97	− 57	112
− 3	−21	112	42	− 79	729	267	− 69	7
− 2	−26	83	227	− 74	− 214	427	− 21	− 98
− 1	−29	44	352	− 44	− 1,012	308	42	− 98
0	−30	0	396	0	− 1,320	0	70	0
570	13,566	213,180	2,288,132	89,148	24,515,700	2,451,570	65,550	164,220
2	3	4	5	84	14	104	1,287	1,430

20

φ_1	φ_2	φ_3	φ_4	φ_5	φ_6	φ_7	φ_8	φ_9
−19	57	− 969	1,938	− 1,938	1,938	− 646	646	− 646
−17	39	− 357	− 102	1,122	− 2,346	1,258	− 1,802	2,414
−15	23	85	− 1,122	1,802	− 1,870	306	510	− 2,006
−13	9	377	− 1,402	1,222	6	− 702	1,422	− 1,586
−11	− 3	539	− 1,187	187	1,497	− 891	549	979
− 9	−13	591	− 687	− 771	1,931	− 387	− 723	1,993
− 7	−21	553	− 77	− 1,351	1,353	321	− 1,239	763
− 5	−27	445	503	− 1,441	195	777	− 735	− 1,127
− 3	−31	287	948	− 1,076	− 988	756	294	− 1,862
− 1	−33	99	1,188	− 396	− 1,716	308	1,078	− 882
2,660	17,556	4,903,140	22,881,320	31,201,800	49,031,400	9,806,280	20,189,400	47,623,800
1	3	1	2	6	14	78	117	143

21

φ_1	φ_2	φ_3	φ_4	φ_5	φ_6	φ_7	φ_8	φ_9
− 10	190	− 285	969	− 3,876	6,460	− 3,230	3,230	− 1,292
− 9	133	− 114	0	1,938	− 7,106	5,814	− 8,398	4,522
− 8	82	12	− 510	3,468	− 6,392	2,006	1,394	− 3,128
− 7	37	98	− 680	2,618	− 918	− 2,754	6,426	− 3,298
− 6	− 2	149	− 615	788	3,996	− 4,266	3,618	932
− 5	− 35	170	− 406	− 1,063	6,075	− 2,565	− 2,025	3,479
− 4	− 62	166	− 130	− 2,354	5,088	543	− 5,421	2,264
− 3	− 83	142	150	− 2,819	2,001	3,087	− 4,557	− 931
− 2	− 98	103	385	− 2,444	− 1,716	3,822	− 588	− 3,136
− 1	−107	54	540	− 1,404	− 4,628	2,548	3,626	− 2,646
0	−110	0	594	0	− 5,720	0	5,390	0
770	201,894	432,630	5,720,330	121,687,020	514,829,700	223,092,870	439,119,450	157,158,540
2	1	4	5	4	6	24	39	130

22

φ_1	φ_2	φ_3	φ_4	φ_5	φ_6	φ_7	φ_8	φ_9
− 21	35	− 133	1,197	− 2,261	646	− 9,690	4,522	− 4,522
− 19	25	− 57	57	969	− 646	16,150	− 10,982	14,858
− 17	16	0	− 570	1,938	− 646	7,106	646	− 8,398
− 15	8	40	− 810	1,598	− 170	− 6,222	7,990	− 11,458
− 13	1	65	− 775	663	303	− 11,934	5,814	442
− 11	− 5	77	− 563	− 363	558	− 8,910	− 954	10,054
− 9	− 10	78	− 258	− 1,158	537	− 1,035	− 6,231	9,139
− 7	− 14	70	70	− 1,554	303	6,717	− 6,783	469
− 5	− 17	55	365	− 1,509	− 30	10,626	− 2,940	− 8,036
− 3	− 19	35	585	− 1,079	− 338	9,282	2,548	− 9,996
− 1	− 20	12	702	− 390	− 520	3,640	6,370	− 4,410
3,542	7,084	96,140	8,748,740	40,562,340	4,903,140	1,848,483,780	761,140,380	1,623,971,580
1	6	10	5	9	84	12	45	63

23

φ_1	φ_2	φ_3	φ_4	φ_5	φ_6	φ_7	φ_8	φ_9
− 11	77	− 77	1,463	− 209	3,553	− 7,106	7,106	− 24,871
− 10	56	− 35	133	76	− 3,230	10,982	− 16,150	76,874
− 9	37	− 3	− 627	171	− 3,553	5,814	− 646	− 34,561
− 8	20	20	− 950	152	− 1,292	− 3,230	10,982	− 60,724
− 7	5	35	− 955	77	1,207	− 7,990	9,758	− 9,469
− 6	− 8	43	− 747	− 12	2,754	− 7,038	918	43,282
− 5	− 19	45	− 417	− 87	2,985	− 2,334	− 7,530	51,637
− 4	− 28	42	− 42	− 132	2,076	3,117	− 10,383	17,752
− 3	− 35	35	315	− 141	501	6,750	− 6,816	− 27,188
− 2	− 40	25	605	− 116	− 1,166	7,210	476	− 50,568
− 1	− 43	13	793	− 65	− 2,405	4,550	7,280	− 38,220
0	− 44	0	858	0	− 2,860	0	10,010	0
1,012	35,420	32,890	13,123,110	340,860	142,191,060	924,241,890	1,685,382,270	42,223,261,08 (1)
2	3	20	5	126	21	24	45	20

24

φ_1	φ_2	φ_3	φ_4	φ_5	φ_6	φ_7	φ_8	φ_9
− 23	253	− 1,771	253	− 4,807	4,807	− 81,719	81,719	− 7,429
− 21	187	− 847	33	1,463	− 3,971	117,249	− 174,097	21,641
− 19	127	− 133	− 97	3,743	− 4,769	72,029	− 22,933	− 7,429
− 17	73	391	− 157	3,553	− 2,147	− 23,579	108,851	− 17,119
− 15	25	745	− 165	2,071	1,045	− 82,3[illegible]5	114,665	− 5,491
− 13	− 17	949	− 137	169	3,271	− 83,317	32,657	9,503
− 11	− 53	1,023	− 87	− 1,551	3,957	− 40,953	− 61,251	14,773
− 9	− 83	987	− 27	− 2,721	3,183	16,767	− 109,419	8,383
− 7	−107	861	33	− 3,171	1,419	63,093	− 92,652	− 3,452
− 5	−125	665	85	− 2,893	− 695	80,845	− 27,340	− 12,372
− 3	−137	419	123	− 2,005	− 2,525	65,625	49,700	− 13,020
− 1	−143	143	143	− 715	− 3,575	25,025	100,100	− 5,460
4,600	394,680	17,760,600	394,680	177,928,920	250,925,400	114,605,994,4 (2)	202,245,872,4 (2)	3,290,124,240
1	1	1	35	7	21	3	6	110

25

φ_1	φ_2	φ_3	φ_4	φ_5	φ_6	φ_7	φ_8	φ_9
− 12	92	− 506	1,518	− 1,012	19,228	− 14,421	22,287	− 1,748
− 11	69	− 253	253	253	− 14,421	19,228	− 44,574	4,807
− 10	48	− 55	− 517	748	− 18,810	13,376	− 9,690	− 1,178
− 9	29	93	− 897	753	− 9,899	− 2,052	25,194	− 3,743
− 8	12	196	− 982	488	2,052	− 12,803	31,008	− 1,748
− 7	− 3	259	− 857	119	11,229	− 14,668	13,566	1,501
− 6	− 16	287	− 597	− 236	15,142	− 9,096	− 10,302	3,166
− 5	− 27	285	− 267	− 501	13,635	− 12	− 26,010	2,411
− 4	− 36	258	78	− 636	8,028	8,409	− 26,793	116
− 3	− 43	211	393	− 631	391	13,092	− 14,136	− 2,124
− 2	− 48	149	643	− 500	− 7,050	12,700	4,800	− 3,000
− 1	− 51	77	803	− 275	− 12,375	7,700	21,000	− 2,100
0	− 52	0	858	0	− 14,300	0	27,300	0
1,300	53,820	1,480,050	14,307,150	7,803,900	3,889,343,700	3,370,764,540	13,789,491,30 (1)	161,280,600
2	3	4	7	42	7	24	33	748

26

φ_1	φ_2	φ_3	φ_4	φ_5	φ_6	φ_7	φ_8	φ_9
− 25	50	− 1,150	2,530	− 2,530	6,325	− 10,925	10,925	− 2,185
− 23	38	− 598	506	506	− 4,301	13,547	− 20,539	5,681
− 21	27	− 161	− 759	1,771	− 6,072	10,488	− 6,118	− 874
− 19	17	171	− 1,419	1,881	− 3,608	− 152	10,298	− 4,294
− 17	8	408	− 1,614	1,326	46	− 8,398	14,744	− 2,584
− 15	0	560	− 1,470	482	3,090	− 10,830	8,360	1,064
− 13	− 7	637	− 1,099	− 377	4,672	− 7,904	− 2,242	3,458
− 11	− 13	649	− 599	− 1,067	4,624	− 1,936	− 10,594	3,278
− 9	− 18	606	− 54	− 1,482	3,231	4,329	− 13,011	1,063
− 7	− 22	518	466	− 1,582	1,033	8,641	− 9,163	− 1,647
− 5	− 25	395	905	− 1,381	− 1,340	9,740	− 1,360	− 3,312
− 3	− 27	247	1,221	− 935	− 3,300	7,500	6,800	− 3,120
− 1	− 28	84	1,386	− 330	− 4,400	2,800	11,900	− 1,260
5,850	16,380	7,803,900	40,060,020	48,384,180	409,404,600	1,838,598,840	3,064,331,400	225,792,840
1	6	2	5	21	28	44	99	935

27

φ_1	φ_2	φ_3	φ_4	φ_5	φ_6	φ_7	φ_8	φ_9
− 13	325	− 130	2,990	− 16,445	1,495	− 7,475	28,405	− 28,405
− 12	250	− 70	690	2,530	− 920	8,625	− 50,255	69,920
− 11	181	− 22	− 782	10,879	− 1,403	7,337	− 18,791	− 4,807
− 10	118	15	− 1,587	12,144	− 920	736	21,850	− 50,692
− 9	61	42	− 1,872	9,174	− 122	− 4,878	36,556	− 37,012
− 8	10	60	− 1,770	4,188	592	− 7,090	24,928	4,712
− 7	− 35	70	− 1,400	− 1,162	1,018	− 5,870	532	37,772
− 6	− 74	73	− 867	− 5,728	1,096	− 2,424	− 21,698	43,244
− 5	−107	70	− 262	− 8,803	865	1,643	− 31,895	22,679
− 4	−134	62	338	− 10,058	424	4,891	− 27,287	− 9,216
− 3	−155	50	870	− 9,479	− 101	6,375	− 11,339	− 34,731
− 2	−170	35	1,285	− 7,304	− 584	5,780	8,704	− 41,616
− 1	−179	18	1,548	− 3,960	− 920	3,400	24,820	− 27,540
0	−182	0	1,638	0	− 1,040	0	30,940	0
1,638	712,530	101,790	56,448,210	2,032,135,560	22,331,160	822,531,060	19,305,287,82 (1)	34,546,304,52 (1)
2	1	20	5	4	154	88	55	110

28

φ_1	φ_2	φ_3	φ_4	φ_5	φ_6	φ_7	φ_8	φ_9
− 27	117	− 585	1,755	− 13,455	13,455	− 40,365	4,485	− 85,215
− 25	91	− 325	455	1,495	− 7,475	43,355	− 7,475	198,835
− 23	67	− 115	− 395	8,395	− 12,305	40,135	− 3,335	2,185
− 21	45	49	− 879	9,821	− 8,763	7,935	2,737	− 136,781
− 19	25	171	− 1,074	7,866	− 2,162	− 21,850	5,428	− 117,116
− 17	7	255	− 1,050	4,182	4,138	− 36,074	4,276	− 9,044
− 15	− 9	305	− 870	22	8,310	− 33,162	940	91,276
− 13	− 23	325	− 590	− 3,718	9,682	− 17,914	− 2,516	125,476
− 11	− 35	319	− 259	− 6,457	8,401	2,365	− 4,559	86,317
− 9	− 45	291	81	− 7,887	5,139	20,565	− 4,551	4,247
− 7	− 53	245	395	− 7,931	841	31,457	− 2,723	− 75,843
− 5	− 59	185	655	− 6,701	− 3,485	32,521	85	− 116,438
− 3	− 63	115	840	− 4,456	− 6,936	24,072	2,788	− 101,388
− 1	− 65	39	936	− 1,560	− 8,840	8,840	4,420	− 39,780
7,308	95,004	2,103,660	19,634,160	1,354,757,040	1,771,605,360	23,030,869,68 (1)	451,585,680	284,047,392,7 (2)
1	3	5	10	6	22	22	495	55

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φ_1	φ_2	φ_3	φ_4	φ_5	φ_6	φ_7	φ_8	φ_9
− 14	126	− 819	4,095	− 8,190	26,910	− 4,485	31,395	− 62,790
− 13	99	− 468	1,170	585	− 13,455	4,485	− 49,335	139,035
− 12	74	− 182	− 780	4,810	− 23,920	4,485	− 25,415	11,960
− 11	51	44	− 1,930	5,885	− 18,285	1,265	14,605	− 89,815
− 10	30	215	− 2,441	4,958	− 6,210	− 1,955	35,305	− 88,366
− 9	11	336	− 2,460	2,946	6,026	− 3,726	31,372	− 20,884
− 8	− 6	412	− 2,120	556	14,832	− 3,754	11,584	51,416
− 7	− 21	448	− 1,540	− 1,694	18,678	− 2,414	− 11,564	86,156
− 6	− 34	449	− 825	− 3,454	17,534	− 381	− 27,827	71,846
− 5	− 45	420	− 66	− 4,521	12,375	1,635	− 31,875	22,567
− 4	− 54	366	660	− 4,818	4,752	3,063	− 23,631	− 34,808
− 3	− 61	292	1,290	− 4,373	− 3,571	3,567	− 7,127	− 74,043
− 2	− 66	203	1,775	− 3,298	− 10,914	3,077	11,407	− 79,458
− 1	− 69	104	2,080	− 1,768	− 15,912	1,768	25,636	− 50,388
0	− 70	0	2,184	0	− 17,680	0	30,940	0
2,030	113,274	4,207,320	107,987,880	500,671,080	6,959,878,200	274,177,020	20,885,837,70 (1)	142,023,696,4 (2)
2	3	4	5	12	14	264	99	110

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φ_1	φ_2	φ_3	φ_4	φ_5	φ_6	φ_7	φ_8	φ_9
− 29	203	− 1,827	23,751	− 16,965	5,655	− 130,065	130,065	− 70,035
− 27	161	− 1,071	7,371	585	− 2,535	121,095	− 192,855	147,315
− 25	122	− 450	− 3,744	9,360	− 4,875	130,065	− 112,125	23,115
− 23	86	46	− 10,504	11,960	− 3,965	46,345	43,355	− 88,435
− 21	53	427	− 13,749	10,535	− 1,655	− 43,815	134,435	− 98,785
− 19	23	703	− 14,249	6,821	823	− 99,199	132,779	− 36,271
− 17	− 4	884	− 12,704	2,176	2,734	− 108,698	64,952	40,664
− 15	− 28	980	− 9,744	− 2,384	3,730	− 79,386	− 24,760	86,744
− 13	− 49	1,001	− 5,929	− 6,149	3,751	− 27,313	− 96,697	84,539
− 11	− 67	957	− 1,749	− 8,679	2,937	29,271	− 126,849	42,229
− 9	− 82	858	2,376	− 9,768	1,551	74,547	− 109,677	− 16,757
− 7	− 94	714	6,096	− 9,408	− 87	97,899	− 55,461	− 65,987
− 5	−103	535	9,131	− 7,753	− 1,655	95,113	15,485	− 86,127
− 3	−109	331	11,271	− 5,083	− 2,873	68,289	79,781	− 70,737
− 1	−112	112	12,376	− 1,768	− 3,536	24,752	117,572	− 21,132
8,990	302,064	21,360,240	3,671,587,920	2,145,733,200	302,603,400	223,180,094,3 (2)	340,140,785,4 (2)	163,873,495,8 (2)
1	2	2	1	7	84	12	33	143

31

φ_1	φ_2	φ_3	φ_4	φ_5	φ_6	φ_7	φ_8	φ_9
− 15	145	− 1,015	783	− 1,131	23,275	− 84,825	50,025	− 10,005
− 14	116	− 609	261	0	− 11,310	73,515	− 70,035	20.010
− 13	89	− 273	− 99	585	− 23,595	84,435	− 45,195	4,485
− 12	64	− 2	− 324	780	− 20,280	35,685	10,695	− 11,040
− 11	41	209	− 439	715	− 9,815	− 20,735	47,035	− 13,915
− 10	20	365	− 467	496	2,050	− 58,675	51,175	− 6,670
− 9	1	471	− 429	207	11,759	− 69,651	30,199	3,611
− 8	− 16	532	− 344	− 88	17,488	− 56,234	− 1,472	10,856
− 7	− 31	553	− 229	− 343	18,727	− 26,869	− 29,813	12,161
− 6	− 44	539	− 99	− 528	15,906	8,019	− 45,309	7,846
− 5	− 55	495	33	− 627	10,065	38,775	− 44,385	415
− 4	− 64	423	156	− 636	2,568	58,263	− 28,977	− 6,848
− 3	− 71	337	261	− 561	− 5,139	62,757	− 4,959	− 11,153
− 2	− 76	233	341	− 416	− 11,726	52,117	19,969	− 11,058
− 1	− 79	119	391	− 221	− 16,133	29,393	38,437	− 6,783
0	− 80	0	408	0	− 17,680	0	45,220	0
2,460	158,224	6,724,520	4,034,712	9,536,592	3,888,399,800	92,183,032,42 (1)	47,968,572,30 (1)	3,121,399,920
2	3	4	35	123	21	24	117	1,430

32

φ_1	φ_2	φ_3	φ_4	φ_5	φ_6	φ_7	φ_8	φ_9
− 31	155	− 899	899	− 2,697	35,061	− 67,425	13,485	− 310,155
− 29	125	− 551	319	− 87	− 12,441	54,375	− 17,835	590.295
− 27	97	− 261	− 87	1,305	− 28,275	66,555	− 12,615	170,085
− 25	71	− 25	− 347	1,815	− 25,545	32,115	1,425	− 295,665
− 23	47	161	− 487	1,725	− 13,845	− 10,695	11,415	− 419,865
− 21	25	301	− 531	1,267	169	− 41,775	13,615	− 212,995
− 19	5	399	− 501	627	12,251	− 53,675	9,235	50,255
− 17	− 13	459	− 417	− 51	20,081	− 47,107	1,531	285,821
− 15	− 29	485	− 297	− 661	22,825	− 27,381	− 6,065	366,551
− 13	− 43	481	− 157	− 1,131	20,739	− 1,677	− 11,001	282,061
− 11	− 55	451	− 11	− 1,419	14,817	22,935	− 12,045	87,175
− 9	− 65	399	129	− 1,509	6,483	40,815	− 9,285	− 132,395
− 7	− 73	329	253	− 1,407	− 2,673	48,501	− 3,843	− 294,083
− 5	− 79	245	353	− 1,137	− 11,115	44,973	2,565	− 344,033
− 3	− 83	151	423	− 737	− 17,537	31,521	8,113	− 270,123
− 1	− 85	51	459	− 255	− 20,995	11,305	11,305	− 101,745
10,912	185,504	5,379,616	5,379,616	54,285,216	11,345,610,14 (1)	56,728,050,72 (1)	3,336,944,160	2,815,502,728 (3)
1	3	5	35	63	21	39	585	65

33

φ_1	φ_2	φ_3	φ_4	φ_5	φ_6	φ_7	φ_8	φ_9
— 16	496	— 248	7,192	— 14,384	43,152	— 10,788	53,940	— 215,760
— 15	403	— 155	2,697	— 899	— 13,485	8,091	— 67,425	391,065
— 14	316	— 77	— 493	6,496	— 33,582	10,527	— 51,765	136,590
— 13	235	— 13	— 2,581	9,425	— 31,755	5,655	435	— 175,305
— 12	160	38	— 3,756	9,260	— 18,840	— 855	40,665	— 281,280
— 11	91	77	— 4,193	7,139	— 2,487	— 5,907	53,085	— 188,265
— 10	28	105	— 4,053	3,984	12,290	— 8,231	40,225	— 3,290
— 9	— 29	123	— 3,483	519	22,607	— 7,767	12,865	162,985
— 8	— 80	132	— 2,616	— 2,712	27,248	— 5,170	— 16,664	240,952
— 7	—125	133	— 1,571	— 5,327	26,247	— 1,425	— 38,451	— 212,747
— 6	—164	127	— 453	— 7,088	20,514	2,439	— 46.875	103,010
— 5	—197	115	647	— 7,883	11,505	5,547	— 40,935	— 39,595
— 4	—224	98	1,652	— 7,708	936	7,299	— 23,535	— 162,400
— 3	—245	77	2,499	— 6,649	— 9,459	7,425	— 153	— 225,211
— 2	—260	53	3,139	— 4,864	— 18,123	5,985	22,743	— 210,406
— 1	—269	27	3,537	— 2,565	— 23,845	3,325	39,235	— 125,685
— 0	—272	0	3,672	0	— 25,840	0	45,220	0
2,992	1,947,792	417,384	348,330,136	1,547,128,656	17,018,415,22 (1)	1,418,201,268	51,305,516,46 (1)	1,285,338,202 (3)
2	1	20	5	14	21	312	195	130

34

φ_1	φ_2	φ_3	φ_4	φ_5	φ_6	φ_7	φ_8	φ_9
— 33	88	— 2,728	8,184	— 79,112	39,556	— 356,004	118,668	— 593,340
— 31	72	— 1,736	3,224	— 7,192	— 10,788	248,124	— 140,244	1,024,860
— 29	57	— 899	— 341	33,263	— 29,667	342,519	— 115,971	418,035
— 27	43	— 207	— 2,721	50,373	— 29,261	201,231	— 9,483	— 404,115
— 25	30	350	— 4,112	51,040	— 18,705	— 2,175	78,735	— 738,195
— 23	18	782	— 4,696	41,032	— 4,551	— 169,671	112,647	— 556,485
— 21	7	1,099	— 4,641	25,037	8,803	— 257,679	93,639	— 101,535
— 19	— 3	1,311	— 4,101	6,897	18,717	— 259,559	41,071	351,215
— 17	— 12	1,428	— 3,216	— 10,608	23,946	— 191,386	— 20,912	610,640
— 15	— 20	1,460	— 2,112	— 25,376	24,310	— 80,730	— 71,520	606,208
— 13	— 27	1,417	— 901	— 36,049	20,397	41,847	— 97,509	374,725
— 11	— 33	1,309	319	— 41,899	13,299	148,863	— 94,413	21,835
— 9	— 38	1,146	1,464	— 42,744	4,381	219,843	— 65,697	— 323,795
— 7	— 42	938	2,464	— 38,864	— 4,917	243,387	— 20,601	— 551,285
— 5	— 45	695	3,263	— 30,917	— 13,245	217,665	28,665	— 595,889
— 3	— 47	427	3,819	— 19,855	— 19,475	149,625	69,825	— 451,335
— 1	— 48	144	4,104	— 6,840	— 22,800	53,200	93,100	— 167,580
13,090	62,832	51,477,360	456,432,592	46,929,569,23 (1)	14,182,012,68 (1)	1,511,802,552 (3)	239,425,743,5 (2)	9,211,590,447 (3)
1	6	2	5	3	28	12	117	65

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φ_1	φ_2	φ_3	φ_4	φ_5	φ_6	φ_7	φ_8	φ_9
−17	187	−1 496	46 376	−23 188	672 452	−672 452	2 017 356	−183 396
−16	154	−968	19 096	−2 728	−158 224	435 116	−2,254,692	302,064
−15	123	−520	−744	9,052	−485,460	636,492	−1,995,780	140,244
−14	94	−147	−14,229	14,322	−498,046	403,651	−320,943	−102,486
−13	67	156	−22,374	14,937	−339,097	41,093	1,162,059	−216,021
−12	42	394	−26,124	12,458	−112,752	−273,963	1,830,219	−180,264
−11	19	572	−26,354	8,173	109,589	−457,391	1,648,887	−56,199
−10	−2	695	−23,869	3,118	283,490	−489,547	882,195	79,746
−9	−21	768	−19,404	−1,902	386,166	−391,806	−107,572	169,876
−8	−38	796	−13,624	−6,292	411,632	−207,818	−988,104	187,736
−7	−53	784	−7,124	−9,646	366,314	10,946	−1,527,708	136,868
−5	−66	737	−429	−11,726	265,122	215,787	−1,622,049	42,038
−5	−77	660	6,006	12,441	127,985	367,939	−1,289,145	−62,377
−4	−86	558	11,796	−11,826	−23,152	442,727	−643,341	−143,512
−3	−93	436	16,626	−10,021	−166,869	431,151	142,443	−178,507
−2	−98	299	20,251	−7,250	−284,350	339,325	878,325	−159,250
−1	−101	152	22,496	−3,800	−361,000	186,200	1,396,500	−93,100
0	−102	0	23,256	0	−387,600	0	1,582,700	0
3,570	290,598	15,775,320	14,834,059,24 (1)	4,045,652,520	4,070,237,639 (3)	5,291,308,931 (3)	66,919,495,30 (4)	837,417,313,3 (2)
2	3	4	1	12	2	8	9	286

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φ_1	φ_2	φ_3	φ_4	φ_5	φ_6	φ_7	φ_8	φ_9
−35	595	−6,545	5,236	−162,316	115,940	−3,362,260	2,139,620	−1,283,772
−33	493	−4,301	2,244	−23,188	−23,188	2,017,356	−2,261,884	2,017,356
−31	397	−2,387	44	58,652	−80,476	3,124,924	−2,132,428	1,046,436
−29	307	−783	−1,476	97,092	−85,684	2,118,044	−492,652	−571,764
−27	223	531	−2,421	104,067	−61,597	412,641	1,052,729	−1,421,319
−25	145	1,575	−2,889	89,685	−25,015	−1,144,775	1,839,905	−1,297,605
−23	73	2,369	−2,971	62,353	12,323	−2,129,731	1,781,789	−542,271
−21	7	2,933	−2,751	28,903	42,881	−2,415,579	1,098,293	367,899
−19	−53	3,287	−2,306	−5,282	62,534	−2,070,734	125,852	1,044,924
−17	−107	3,451	−1,706	−36,142	69,842	−1,275,646	−807,844	1,282,004
−15	−155	3,445	−1,014	−60,814	65,390	−257,790	−1,456,780	1,059,812
−13	−197	3,289	−286	−77,506	51,194	757,042	−1,688,908	502,892
−11	−233	3,003	429	−85,371	30,173	1,578,863	−1,488,331	−186,043
−9	−263	2,607	1,089	−84,381	5,687	2,074,743	−936,739	−793,633
−7	−287	2,121	1,659	−75,201	−18,859	2,178,907	−182,287	−1,151,563
−5	−305	1,565	2,111	−59,063	−40,345	1,893,395	597,065	−1,172,521
−3	−317	959	2,424	−37,640	−56,200	1,281,000	1,229,900	−863,380
−1	−323	323	2,584	−12,920	−64,600	452,200	1,582,700	−316,540
15,540	3,011,652	307,618,740	191,407,216	199,046,104,0 (2)	120,302,590,3 (2)	130,015,019,4 (5)	73,003,085,78 (4)	39,191,130,26 (4)
1	1	1	10	2	14	2	11	55

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φ_1	φ_2	φ_3	φ_4	φ_5	φ_6	φ_7	φ_8	φ_9
− 18	210	− 357	11,781	− 4,488	139,128	− 94,860	61,132	− 855,848
− 17	175	− 238	5,236	− 748	− 23,188	52,700	− 61,132	1,283,772
− 16	142	− 136	374	1,496	− 92,752	86,428	− 61,132	733,584
− 15	111	− 50	− 3,036	2,596	− 102,300	62,124	− 17,980	− 291,276
− 14	82	21	− 5,211	2,856	− 77,128	16,988	25,172	− 884,616
− 13	55	78	− 6,354	2,535	− 36,115	− 26,195	49,445	− 876,525
− 12	30	122	− 6,654	1,850	7,320	− 55,455	51,185	− 445,440
− 11	7	154	− 6,286	979	44,327	− 66,583	35,177	127,281
− 10	− 14	175	− 5,411	64	69,800	− 60,584	9,770	596,316
− 9	− 33	186	− 4,176	− 786	81,618	− 41,598	− 16,412	815,156
− 8	− 50	188	− 2,714	− 1,492	79,952	− 15,250	− 36,472	747,688
− 7	− 65	182	− 1,144	− 2,002	66,638	12,610	− 46,228	448,708
− 6	− 78	169	429	− 2,288	44,616	36,816	− 44,434	29,068
− 5	− 89	150	1,914	− 2,343	17,435	53,483	− 32,455	− 382,817
− 4	− 98	126	3,234	− 2,178	− 11,176	60,331	− 13,591	− 674,912
− 3	−105	98	4,326	− 1,819	− 37,671	56,775	7,781	− 775,859
− 2	−110	67	5,141	− 1,304	− 58,984	43,820	27,104	− 667,184
− 1	−113	34	5,644	− 680	− 72,760	23,800	40,460	− 383,180
0	−114	0	5,814	0	− 77,520	0	45,220	0
4,218	383,838	932,178	980,961,982	152,877,192	172,433,712,8 (2)	101,892,648,5 (2)	57,938,956,97 (1)	16,692,518,45 (4)
2	3	20	5	84	14	88	495	110

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φ_1	φ_2	φ_3	φ_4	φ_5	φ_6	φ_7	φ_8	φ_9
− 37	111	− 777	1,887	− 20,757	7,548	− 77,996	77,996	−2,261,884
− 35	93	− 525	867	− 3,927	− 1,020	40,052	− 73,780	3,239,996
− 33	76	− 308	102	6,358	− 4,828	69,564	− 77,996	2,017,356
− 31	60	− 124	− 442	11,594	− 5,508	52,700	− 27,404	− 550,188
− 29	45	29	− 797	13,079	− 4,332	17,980	26,164	−2,168,388
− 27	31	153	− 993	11,925	− 2,260	− 16,740	58,900	−2,319,420
− 25	18	250	− 1,058	9,070	15	− 41,695	64,945	−1,361,985
− 23	6	322	− 1,018	5,290	2,025	− 53,015	48,745	50,025
− 21	− 5	371	− 897	1,211	3,488	− 50,880	19,474	1,307,958
− 19	− 15	399	− 717	− 2,679	4,272	− 38,000	− 12,814	2,013,658
− 17	− 24	408	− 498	− 6,018	4,362	− 18,394	− 39,616	2,024,224
− 15	− 32	400	− 258	− 8,558	3,830	3,558	− 55,280	1,420,784
− 13	− 39	377	− 13	− 10,153	2,808	23,816	− 57,434	436,514
− 11	− 45	341	223	− 10,747	1,464	39,160	− 46,778	− 629,266
− 9	− 50	294	438	− 10,362	− 19	47,475	− 26,447	−1,491,601
− 7	− 54	238	622	− 9,086	− 1,461	47,867	− 1,127	−1,942,327
− 5	− 57	175	767	− 7,061	− 2,700	40,636	23,920	−1,887,472
− 3	− 59	107	867	− 4,471	− 3,604	27,132	43,792	−1,357,552
− 1	− 60	36	918	− 1,530	− 4,080	9,520	54,740	− 492,660
18,278	109,668	4,496,388	25,479,532	3,286,859,628	505,670,712	67,928,432,31 (1)	91,903,173,13 (1)	112,078,338,1 (5)
1	6	10	35	21	308	132	495	55

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φ_1	φ_2	φ_3	φ_4	φ_5	φ_6	φ_7	φ_8	φ_9
−19	703	−2,109	2,109	−35,853	11,951	−47,804	1,481,924	−740,962
−18	592	−1,443	999	−7,548	−1,258	22,644	−1,325,932	1,013,948
−17	487	−867	159	10,047	−7,327	41,684	−1,477,708	681,938
−16	388	−376	−446	19,312	−8,636	33,116	−596,564	−113,832
−15	295	35	−849	22,321	−7,055	13,260	389,980	−654,534
−14	208	371	−1,081	20,860	−4,010	−7,460	1,037,260	−753,300
−13	127	637	−1,171	16,445	−545	−23,140	1,215,820	−495,690
−12	52	838	−1,146	10,340	2,620	−31,215	982,855	−68,820
−11	−17	979	−1,031	3,575	5,035	−31,460	487,090	341,715
−10	−80	1,065	−849	−3,036	6,470	−25,160	−98,170	604,894
−9	−137	1,101	−621	−8,877	6,869	−14,436	−618,694	662,197
−8	−188	1,092	−366	−13,512	6,308	−1,714	−964,168	522,652
−7	−233	1,043	−101	−16,667	4,957	10,676	−1,078,322	244,817
−6	−272	959	159	−18,212	3,046	20,784	−958,046	−86,698
−5	−305	845	401	−18,143	835	27,220	−644,810	−384,553
−4	−332	706	614	−16,564	−1,412	29,243	−211,313	−577,996
−3	−353	547	789	−13,669	−3,449	26,772	254,104	−625,876
−2	−368	373	919	−9,724	−5,066	20,332	663,136	−522,376
−1	−377	189	999	−5,049	−6,103	10,948	941,528	−295,596
0	−380	0	1,026	0	−6,460	0	1,040,060	0
4,940	4,496,388	33,722,910	32,224,114	9,860,578,884	1,264,176,780	25,199,257,15 (1)	32,395,868,53 (4)	11,594,310,84 (4)
2	1	4	35	14	231	264	33	220

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φ_1	φ_2	φ_3	φ_4	φ_5	φ_6	φ_7	φ_8	φ_9
−39	247	−9,139	82,251	−9,139	155,363	−466,089	310,726	−9,632,506
−37	209	−6,327	40,071	−2,109	−11,951	203,167	−262,922	12,596,354
−35	173	−3,885	7,881	2,331	−91,205	396,899	−308,210	9,086,534
−33	139	−1,793	−15,579	4,741	−110,959	329,307	−139,298	−684,046
−31	107	−31	−31,499	5,611	−93,823	149,141	60,554	−7,788,006
−29	77	1,421	−40,999	5,365	−57,205	−46,835	200,090	−9,628,290
−27	49	2,583	−45,129	4,365	−14,015	−202,095	249,170	−6,959,190
−25	23	3,475	−44,869	2,915	26,675	−290,215	214,850	−1,890,690
−23	−1	4,117	−41,129	1,265	59,015	−306,245	123,455	3,347,535
−21	−23	4,529	−34,749	−385	79,805	−259,485	7,735	7,074,085
−19	−43	4,731	−26,499	−1,881	87,967	−167,561	−101,741	8,417,551
−17	−61	4,743	−17,079	−3,111	84,061	−51,697	−181,733	7,306,141
−15	−77	4,585	−7,119	−4,001	69,845	66,921	−218,735	4,293,961
−13	−91	4,277	2,821	−4,511	47,879	169,793	−209,287	309,491
−11	−103	3,839	12,251	−4,631	21,173	242,869	−158,779	−3,609,199
−9	−113	3,291	20,751	−4,377	−7,121	277,533	−79,267	−6,546,733
−7	−121	2,653	27,971	−3,787	−33,973	270,911	13,244	−7,881,028
−5	−127	1,945	33,631	−2,917	−56,695	225,607	101,660	−7,377,388
−3	−131	1,187	37,521	−1,837	−73,117	148,971	170,476	−5,203,428
−1	−133	399	39,501	−627	−81,719	52,003	208,012	−1,872,108
21,320	567,112	644,482,280	49,625,135,56 (1)	644,482,280	213,224,483,6 (2)	2,368,730,172 (3)	1,393,370,689 (3)	1,893,737,437 (6)
1	3	1	1	63	21	33	198	22

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φ_1	φ_2	φ_3	φ_4	φ_5	φ_6	φ_7	φ_8	φ_9
−20	260	−2,470	18,278	−36,556	182,780	−776,815	776,815	−1,242,904
−19	221	−1,729	9,139	−9,139	−9,139	310,726	−621,452	1,553,630
−18	184	−1,083	2,109	8,436	−102,638	645,354	−764,864	1,195,100
−17	149	−527	−3,071	18,241	−128,797	557,294	−379,916	6,290
−16	116	−56	−6,646	22,096	−112,396	278,596	101,422	−913,240
−15	85	335	−8,847	21,583	−72,685	−37,230	456,620	−1,212,678
−14	56	651	−9,891	18,060	−24,110	−298,010	604,520	−949,620
−13	29	897	−9,981	12,675	23,005	−457,990	552,140	−358,410
−12	4	1,078	−9,306	6,380	61,820	−505,065	354,335	296,760
−11	−19	1,199	−8,041	−55	88,385	−450,010	84,430	804,595
10	−40	1,265	−6,347	−6,028	101,090	−317,570	−185,930	1,042,798
−9	−59	1,281	−4,371	−11,091	100,159	−139,266	−399,338	982,165
−8	−76	1,252	−2,246	−14,936	87,188	52,226	−519,086	670,660
−7	−91	1,183	−91	−17,381	64,727	227,266	−530,894	207,025
−6	−104	1,079	1,989	−18,356	35,906	362,154	−441,142	−288,730
−5	−115	945	3,903	−17,889	4,105	440,890	−272,710	−702,601
−4	−124	786	5,574	−16,092	−27,332	455,933	−59,401	−948,040
−3	−131	607	6,939	−13,147	−55,339	408,102	160,208	−979,540
−2	−136	413	7,949	−9,292	−77,326	305,762	348,772	−797,640
−1	−139	209	8,569	−4,807	−91,333	163,438	475,456	−445,740
0	−140	0	8,778	0	−96,140	0	520,030	0
5,740	641,732	47,900,710	2,481,256,778	10,376,164,71 (1)	294,751,492,0 (2)	6,514,007,973 (3)	8,534,395,472 (3)	30,544,152,22 (4)
2	3	4	5	18	21	24	99	220

42

φ_1	φ_2	φ_3	φ_4	φ_5	φ_6	φ_7	φ_8	φ_9
−41	410	−1,066	20,254	−749,398	374,699	−1,873,495	6,369,883	−374,699
−39	350	−754	10,374	−201,058	−9,139	685,425	−4,816,253	447,811
−37	293	−481	2,717	155,363	−201,058	1,517,074	−6,214,520	365,560
−35	239	−245	−2,983	359,233	−260,110	1,359,602	−3,346,280	28,120
−33	188	−44	−6,978	445,258	−233,692	737,484	446,590	−248,270
−31	140	124	−9,506	443,734	−158,932	3,100	3,389,086	−354,578
−29	95	261	−10,791	380,799	−63,998	−626,690	4,779,584	−297,888
−27	53	369	−11,043	278,685	30,670	−1,038,690	4,600,880	−138,480
−25	14	450	−10,458	155,970	111,205	−1,195,945	3,222,035	50,925
−23	−22	506	−9,218	27,830	169,235	−1,114,465	1,178,155	208,955
−21	−55	539	−7,491	−93,709	200,882	−843,810	−983,906	296,858
−19	−85	551	−5,431	−199,519	205,838	−451,250	−2,805,074	300,998
−17	−112	544	−3,178	−283,118	186,518	−9,214	−3,969,110	229,670
−15	−136	520	−858	−340,418	147,290	414,258	−4,322,630	106,730
−13	−157	481	1,417	−369,473	93,782	762,346	−3,868,670	−36,010
−11	−175	429	3,549	−370,227	32,266	993,850	−2,741,518	−166,166
−9	−190	366	5,454	−344,262	−30,881	1,085,445	−1,169,677	−256,931
−7	−202	294	7,062	−294,546	−89,639	1,032,077	567,035	−291,365
−5	−211	215	8,317	−225,181	−138,730	845,786	2,181,100	−264,540
−3	−217	131	9,177	−141,151	−173,926	553,242	3,417,340	−183,540
−1	−220	44	9,614	−48,070	−192,280	192,280	4,085,950	−65,550
24,682	1,629,012	9,075,924	3,084,805,724	4,389,117,671 (3)	1,237,956,266 (3)	37,551,340,08 (4)	563,270,101,2 (5)	2,695,072,254 (3)
1	2	10	5	1	12	12	15	935

43

φ_1	φ_2	φ_3	φ_4	φ_5	φ_6	φ_7	φ_8	φ_9
−21	287	−574	22,386	−70,889	374,699	−1,124,097	2,622,893	−201,761
−20	246	−410	11,726	−20,254	0	374,699	−1,873,495	230,584
−19	207	−266	3,406	13,091	−191,919	886,483	−2,531,503	198,949
−18	170	−141	−2,847	32,604	−255,892	822,510	−1,462,240	28,120
−17	135	−34	−7,292	41,344	−236,208	478,040	35,150	−119,510
−16	102	56	−10,174	41,992	−167,832	54,316	1,251,266	−184,112
−15	71	130	−11,724	36,872	−77,560	−322,032	1,886,270	−164,762
−14	42	189	−12,159	27,972	14,892	−582,014	1,906,744	−88,872
−13	15	234	−11,682	16,965	95,865	−699,595	1,434,865	7,995
−12	−10	266	−10,482	5,230	156,800	−679,155	665,465	94,280
−11	−33	286	−8,734	−6,127	193,347	−544,951	−193,171	148,577
−10	−54	295	−6,599	−16,252	204,540	−332,438	−958,630	161,492
−9	−73	294	−4,224	−24,522	192,038	−81,306	−1,497,394	134,642
−8	−90	284	−1,742	−30,524	159,432	169,910	−1,734,850	77,960
−7	−105	266	728	−34,034	111,618	387,790	−1,655,290	6,230
−6	−118	241	3,081	−34,996	54,236	546,702	−1,294,618	−64,444
−5	−129	210	5,226	−33,501	−6,825	630,331	−728,195	−119,729
−4	−138	174	7,086	−29,766	−65,856	632,207	−55,967	−149,384
−3	−145	134	8,598	−24,113	−117,691	555,375	613,265	−148,635
−2	−150	91	9,713	−16,948	−158,004	411,350	1,176,860	−118,560
−1	−153	46	10,396	−8,740	−183,540	218,500	1,551,350	−65,550
0	−154	0	10,626	0	−192,280	0	1,682,450	0
6,622	814,506	2,676,234	3,815,417,606	39,541,600,64 (1)	1,237,956,266 (3)	13,411,192,89 (4)	93,878,350,20 (4)	760,148,584,6 (2)
2	3	20	5	12	14	24	45	2,210

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φ_1	φ_2	φ_3	φ_4	φ_5	φ_6	φ_7	φ_8	φ_9
−43	301	−12,341	12,341	−22,919	435,461	−16,112,057	1,239,389	−8,675,723
−41	259	−8,897	6,601	−6,929	10,127	4,871,087	−835,867	9,482,767
−39	219	−5,863	2,091	3,731	−212,667	12,365,067	−1,181,743	8,618,077
−37	181	−3,219	−1,329	10,101	−292,201	11,853,283	−726,199	1,721,647
−35	145	−945	−3,792	13,104	−276,640	7,311,200	−49,210	−4,548,410
−33	111	979	−5,424	13,552	−204,288	1,484,736	524,438	−7,593,806
−31	79	2,573	−6,344	12,152	−104,776	−3,863,096	849,446	−7,200,866
−29	49	3,857	−6,664	9,512	−184	−7,735,576	899,414	−4,352,726
−27	21	4,851	−6,489	6,147	93,903	−9,713,979	719,497	−431,151
−25	−5	5,575	−5,917	2,485	167,405	−9,795,875	390,545	3.277,475
−23	−29	6,049	−5,039	−1,127	214,823	−8,258,311	3,077	5,845,841
−21	−51	6,293	−3,939	−4,417	234,381	−5,544,159	−360,451	6,802,091
−19	−71	6,327	−2,694	−7,182	227,234	−2,169,914	−636,514	6,111,806
−17	−89	6,171	−1,374	−9,282	196,742	1,346,774	−785,842	4,089,146
−15	−105	5,845	−42	−10,634	147,810	4,541,550	−794,410	1,272,110
−13	−119	5,369	1,246	−11,206	86,294	7,041,502	−671,194	−1,710,982
−11	−131	4,763	2,441	−11,011	18,473	8,588,723	−443,683	−4,263,787
−9	−141	4,047	3,501	−10,101	−49,413	9,051,003	−152,027	−5,919,377
−7	−149	3,241	4,391	−8,561	−111,559	8,421,367	157,409	−6,404,787
−5	−155	2,365	5,083	−6,503	−162,925	6,808,175	438,425	−5,669,505
−3	−159	1,439	5,556	−4,060	−199,500	4,417,500	650,750	−3,878,850
−1	−161	483	5,796	−1,380	−218,500	1,529,500	764,750	−1,376,550
28,380	913,836	1,257,829,980	1,173,974,648	4,162,273,752	1,672,913,873 (3)	2,735,883,349 (6)	20,632,604,44 (4)	1,369,787,749 (6)
1	3	1	10	42	14	2	117	65

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φ_1	φ_2	φ_3	φ_4	φ_5	φ_6	φ_7	φ_8	φ_9
— 22	946	— 3,311	19,393	— 38,786	504,218	— 368,467	13,633,279	— 27,266,558
— 21	817	— 2,408	10,578	— 12,341	22,919	100,491	— 8,675,723	28,505,947
— 20	694	— 1,610	3,608	5,494	— 234,520	274,987	— 12,826,235	27,208,912
— 19	577	— 912	— 1,722	16,359	— 332,059	271,871	— 8,329,847	6,888,697
— 18	466	— 309	— 5,607	21,714	— 321,958	176,643	— 1,218,299	— 12,503,558
— 17	361	204	— 8,232	22,848	— 246,064	49,096	5,051,758	— 22,778,606
— 16	262	632	— 9,772	20,888	— 137,032	— 71,668	8,856,394	— 22,799,696
— 15	169	980	— 10,392	16,808	— 19,480	— 162,816	9,806,110	— 15,104,066
— 14	82	1,253	— 10,247	11,438	88,922	— 213,923	8,274,301	— 3,595,946
— 13	1	1,456	— 9,482	5,473	176,429	— 223,639	5,036,137	7,916,519
— 12	— 74	1,594	— 8,232	— 518	236,264	— 196,851	1,002,517	16,538,096
— 11	—143	1,672	— 6,622	— 6,083	265,727	— 142,307	— 2,965,259	20,626,661
— 10	—206	1,695	— 4,767	— 10,878	265,370	— 70,669	— 6,173,465	19,800,206
— 9	—263	1,668	— 2,772	— 14,658	238,238	7,038	— 8,158,946	14,729,786
— 8	—314	1,596	— 732	— 17,268	189,176	80,614	— 8,708,522	6,815,096
— 7	—359	1,484	1,268	— 18,634	124,202	141,542	— 7,845,194	— 2,181,202
— 6	—398	1,337	3,153	— 18,754	49,946	183,549	— 5,790,557	— 10,482,982
— 5	—431	1,160	4,858	— 17,689	— 26,845	202,903	— 2,911,835	— 16,593,397
— 4	—458	958	6,328	— 15,554	— 99,736	198,479	339,037	— 19,505,232
— 3	—479	736	7,518	— 12,509	— 162,967	171,627	3,487,849	— 18,816,327
— 2	—494	499	8,393	— 8,750	— 211,750	125,875	6,096,625	14,748,750
— 1	—503	252	8,928	— 4,500	— 242,500	66,500	7,813,750	— 8,079,750
0	—506	0	9,108	0	— 253,000	0	8,412,250	0
7,590		92,036,340		12,006,558,90 (1)		1,421,976,792 (3)		13,208,667,58 (7)
	9,203,634		2,934,936,620		2,245,226,514 (3)		2,460,438,079 (6)	
2	1	4	7	28	14	104	13	26

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φ_1	φ_2	φ_3	φ_4	φ_5	φ_c	φ_7	φ_8	φ_9
— 45	165	— 7,095	4,257	— 58,179	290,895	— 872,685	1,842,335	— 1,239,389
— 43	143	— 5,203	2,365	— 19,393	19,393	213,323	— 1,105,401	1,239,389
— 41	122	— 3,526	860	7,052	— 128,699	632,917	— 1,708,347	1,239,389
— 39	102	— 2,054	— 300	23,452	— 187,821	644,397	— 1,166,163	374,699
— 37	83	— 777	— 1,155	31,857	— 186,263	438,413	— 2 50,059	— 489,991
— 35	65	315	— 1,743	34,083	— 146,825	149,845	588,525	— 983,497
— 33	48	1,232	— 2,100	31,724	— 87,444	— 131,604	1,128,258	— 1,036,222
— 31	32	1,984	— 2,260	26,164	— 21,788	— 352,036	1,305,186	— 740,962
— 29	17	2,581	— 2,255	18,589	40,183	— 485,141	1,154,949	— 256,447
— 27	3	3,033	— 2,115	9,999	91,689	— 525,069	768,077	255,183
— 25	— 10	3,350	— 1,868	1,220	128,635	— 480,325	256,605	664,645
— 23	— 22	3,542	— 1,540	— 7,084	149,149	— 368,621	— 269,643	892,147
— 21	— 33	3,619	— 1,155	— 14,399	153,153	— 212,619	— 718,641	910,027
— 19	— 43	3,591	— 735	— 20,349	141,967	— 36,499	— 1,025,049	736,117
— 17	— 52	3,468	— 300	— 24,684	117,946	136,714	— 1,153,722	421,702
— 15	— 60	3,260	132	— 27,268	84,150	287,130	— 1,099,050	37,162
— 13	— 67	2,977	545	— 28,067	44,047	399,347	— 881,229	— 342,329
— 11	— 73	2,629	925	— 27,137	1,249	463,243	— 540,453	— 649,319
— 9	— 78	2,226	1,260	— 24,612	— 40,719	474,273	— 129,907	— 833,659
— 7	— 82	1,778	1,540	— 20,692	— 78,617	433,337	291,669	— 868,509
— 5	— 85	1,295	1,757	— 15,631	— 109,655	346,285	667,185	— 752,571
— 3	— 87	787	1,905	— 9,725	— 131,625	223,125	947,625	— 508,725
— 1	— 88	264	1,980	3,300	— 143,000	77,000	1,097,250	— 179,550
32,430		429,502,920		27,214,866,84 (1)		7,933,133,684 (3)		26,684,176,94 (4)
	285,384		143,167,640		748,408,838,1 (2)		44,332,217,65 (4)	
1	6	2	35	21	28	52	117	715

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φ_1	φ_2	φ_3	φ_4	φ_5	φ_6	φ_7	φ_8	φ_9
− 23	345	− 759	32,637	− 32,637	1,338,117	− 2,230,195	40,549	− 770,431
− 22	300	− 561	18,447	− 11,352	116,358	484,825	− 22,919	736,934
− 21	257	− 385	7,095	3,311	− 562,397	1,570,833	− 37,023	770,431
− 20	216	− 230	− 1,720	12,556	− 846,240	1,644,879	− 26,445	267,976
− 19	177	− 95	− 8,285	17,461	− 857,433	1,166,163	− 7,257	− 257,849
− 18	140	21	− 12,873	18,984	− 695,114	463,095	10,947	− 578,018
− 17	105	119	− 15,743	17,969	− 437,871	− 242,505	23,283	− 639,863
− 16	72	200	− 17,140	15,152	− 146,184	− 813,924	28,134	− 488,528
− 15	41	265	− 17,295	11,167	135,265	− 1,180,647	25,995	− 211,793
− 14	12	315	− 16,425	6,552	375,414	− 1,321,879	18,571	96,402
− 13	− 15	351	− 14,733	1,755	554,775	− 1,252,355	8,095	357,825
− 12	− 40	374	− 12,408	− 2,860	663,520	− 1,010,295	− 3,165	520,760
− 11	− 63	385	− 9,625	− 7,007	699,699	− 647,361	− 13,239	562,793
− 10	− 84	385	− 6,545	− 10,472	667,590	− 220,473	− 20,655	488,138
− 9	−103	375	− 3,315	− 13,107	576,181	214,659	− 24,531	321,623
− 8	−120	356	− 68	− 14,824	437,784	608,430	− 24,582	101,048
− 7	−135	329	3,077	− 15,589	266,781	920,805	− 21,063	− 130,747
− 6	−148	295	6,015	− 15,416	78,502	1,123,497	− 14,667	− 333,106
− 5	−159	255	8,655	− 14,361	− 111,765	1,201,001	− 6,395	− 473,381
− 4	−168	210	10,920	− 12,516	− 289,632	1,150,627	2,587	− 530,936
− 3	−175	161	12,747	− 10,003	− 442,337	981,675	11,091	− 499,149
− 2	−180	109	14,087	− 6,968	− 559,338	713,895	18,039	− 385,434
− 1	−183	55	14,905	− 3,575	− 632,775	375,375	22,575	− 209,475
0	−184	0	15,180	0	− 657,800	0	24,150	0
8,648	1,271,256	4,994,220	8,518,474,580	8,629,104,120	15,866,267,37 (4)	51,565,368,95 (4)	21,211,587,39 (1)	10,096,715,60 (4)
2	3	20	5	42	7	24	6,435	1,430

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φ_1	φ_2	φ_3	φ_4	φ_5	φ_6	φ_7	φ_8	φ_9
− 47	1,081	− 3,243	35,673	−1,533,939	511,313	−1,905,803	1,905,803	− 1,905,803
− 45	943	− 2,415	20,493	− 554,829	54,395	364,941	− 1,013,725	1,743,607
− 43	811	− 1,677	8,283	126,291	− 203,863	1,302,857	− 1,711,873	1,902,277
− 41	685	− 1,025	− 1,265	562,397	− 316,437	1,401,585	− 1,274,649	742,223
− 39	565	− 455	− 8,445	801,047	− 327,273	1,031,355	− 417,831	− 527,137
− 37	451	37	− 13,537	884,633	− 272,211	459,651	423,489	− 1,345,579
− 35	343	455	− 16,807	850,633	− 179,865	− 130,257	1,020,285	− 1,563,289
− 33	241	803	− 18,507	731,863	− 72,459	− 622,809	1,288,341	− 1,264,219
− 31	145	1,085	− 18,875	556,729	33,381	− 955,575	1,238,877	− 640,429
− 29	55	1,305	− 18,135	349,479	125,879	−1,106,495	939,653	92,481
− 27	− 29	1,467	− 16,497	130,455	197,405	−1,082,835	485,225	747,555
− 25	−107	1,575	− 14,157	− 83,655	243,815	− 911,755	− 24,895	1,193,865
− 23	−179	1,633	− 11,297	− 279,565	263,835	− 632,385	− 501,345	1,365,625
− 21	−245	1,645	− 8,085	− 447,139	258,489	− 289,305	− 874,377	1,258,579
− 19	−305	1,615	− 4,675	− 579,139	230,571	72,675	− 1,098,693	918,289
− 17	−359	1,547	− 1,207	− 670,973	184,161	412,539	− 1,154,589	424,099
− 15	−407	1,445	2,193	− 720,443	124,185	695,997	− 1,046,265	− 128,231
− 13	−449	1,313	5,413	− 727,493	56,019	897,429	− 798,081	− 642,941
− 11	−485	1,155	8,355	− 693,957	− 14,863	1,000,945	− 449,461	− 1,038,103
− 9	−515	975	10,935	− 623,307	− 83,197	1,000,665	− 49,069	− 1,255,813
− 7	−539	777	13,083	− 520,401	− 144,193	900,323	351,197	− 1,268,197
− 5	−557	565	14,743	− 391,231	− 193,755	712,299	701,925	− 1,079,127
− 3	−569	343	15,873	− 242,671	− 228,657	456,183	961,233	− 721,917
− 1	−575	115	16,445	− 82,225	− 246,675	156,975	1,098,825	− 253,575
36,848	12,712,560	92,620,080	10,301,411,12(1)	19,208,385,77 (4)	2,321,892,786 (3)	37,502,086,51 (4)	46,326,106,86 (4)	60,580,293,59 (4)
1	1	5	5	1	21	33	165	715

49

φ_1	φ_2	φ_3	φ_4	φ_5	φ_6	φ_7	φ_8	φ_9
− 24	376	− 4,324	38,916	− 95,128	371,864	− 278,898	3,811,606	− 15,246,424
− 23	329	− 3,243	22,701	− 35,673	46,483	46,483	− 1,905,803	13,340,621
− 22	284	− 2,277	9,591	6,072	− 140,438	184,943	− 3,365,567	15,165,326
− 21	241	− 1,421	− 729	33,187	− 225,019	204,207	− 2,603,951	6,517,811
− 20	200	− 670	− 8,560	48,444	− 237,360	155,445	− 978,465	− 3,370,856
− 19	161	− 19	− 14,189	54,321	− 202,143	75,981	671,703	− 10,082,597
− 18	124	537	− 17,889	53,016	− 139,206	− 8,271	1,891,371	− 12,288,602
− 17	89	1,003	− 19,919	46,461	− 64,089	− 80,631	2,498,499	− 10,462,667
− 16	56	1,384	− 20,524	36,336	11,448	− 131,724	2,494,242	− 5,955,152
− 15	25	1,685	− 19,935	24,083	78,935	− 157,785	1,991,515	− 359,517
− 14	− 4	1,911	− 18,369	10,920	132,730	− 159,185	1,159,795	4,888,650
− 13	− 31	2,067	− 16,029	− 2,145	169,585	− 139,165	184,015	8,732,685
− 12	− 56	2,158	− 13,104	− 14,300	188,240	− 102,765	− 764,465	10,581,480
− 11	− 79	2,189	− 9,769	− 24,915	189,045	− 55,935	− 1,546,695	10,300,565
− 10	− 100	2,165	− 6,185	− 33,528	173,610	− 4,815	− 2,066,535	8,140,322
− 9	− 119	2,091	− 2,499	− 39,831	144,483	44,829	− 2,274,243	4,628,267
− 8	− 136	1,972	1,156	− 43,656	104,856	88,026	− 2,164,746	447,032
− 7	− 151	1,813	4,661	− 44,961	58,299	120,891	− 1,771,959	− 3,685,183
− 6	− 164	1,619	7,911	− 43,816	8,522	140,799	− 1,160,387	− 7,119,338
− 5	− 175	1,395	10,815	− 40,389	− 40,835	146,455	− 415,115	− 9,357,089
− 4	− 184	1,146	13,296	− 34,932	− 86,384	137,873	368,839	− 10,104,296
− 3	− 191	877	15,291	− 27,767	− 125,143	116,277	1,096,963	− 9,294,761
− 2	− 196	593	16,751	− 19,272	− 154,662	83,937	1,684,767	− 7,085,106
− 1	− 199	299	17,641	− 9,867	− 173,121	43,953	2,065,791	− 3,823,911
0	− 200	0	17,940	0	− 179,400	0	2,197,650	0
9,800	1,566,040	167,230,700	12,408,517,94(1)	74,451,107,64 (1)	1,231,306,780 (3)	800,349,407,1 (2)	18 3.374,173,0(5)	3,806,461,780 (6)
2	3	4	5	18	33	264	99	110

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φ_1	φ_2	φ_3	φ_4	φ_5	φ_6	φ_7	φ_8	φ_9
− 49	196	− 9,212	211,876	− 211,876	15,134	− 650,762	4,555,334	186,768,694
− 47	172	− 6,956	125,396	− 82,156	2,162	92,966	− 2,138,218	156,275·846
− 45	149	− 4,935	55,131	9,729	− 5,405	418,347	− 3,951,055	184,862,891
− 43	127	− 3,139	− 529	70,219	− 8,947	473,731	− 3,167,767	86,328,821
− 41	106	− 1,558	− 43,124	105,124	− 9,619	371,993	− 1,327,969	− 31,404,319
− 39	86	− 182	− 74,124	119,652	− 8,373	196,209	601,527	− 115,122,137
− 37	67	999	− 94,929	118,437	− 5,979	4,773	2,081,931	− 147,226,367
− 35	49	1,995	− 106,869	105,567	− 3,045	− 164,019	2,882,355	− 131,363,057
− 33	32	2,816	− 111,204	84,612	− 36	− 287,892	2,981,754	− 81,965,642
− 31	16	3,472	− 109,124	58,652	2,708	− 356,996	2,490,458	− 16,994,262
− 29	1	3,973	− 101,749	30,305	4,955	− 370,765	1,588,955	46,765,545
− 27	− 13	4,329	− 90,129	1,755	6,565	− 335,205	481,715	96,404,295
− 25	− 26	4,550	− 75,244	− 25,220	7,475	− 260,585	− 636,025	124,076,745
− 23	− 38	4,646	− 58,004	− 49,220	7,685	− 159,505	− 1,600,465	127,094,895
− 21	− 49	4,627	− 39,249	− 69,195	7,245	− 45,315	− 2,292,255	107,279,795
− 19	− 59	4,503	− 19,749	− 84,417	6,243	69,141	− 2,642,007	69,866,477
− 17	− 68	4,284	− 204	− 94,452	4,794	172,482	− 2,629,866	22,201,082
− 15	− 76	3,980	18,756	− 99,132	3,030	255,474	− 2,280,570	− 27,581,578
− 13	− 83	3,601	36,571	− 98,527	1,091	311,467	− 1,655,299	− 71,758,193
− 11	− 89	3,157	52,751	− 92,917	− 883	336,611	− 841,483	− 103,995,023
− 9	− 94	2,658	66,876	− 82,764	− 2,759	329,877	58,391	− 120,041,591
− 7	− 98	2,114	78,596	− 68,684	− 4,417	292,909	938,063	− 118,110,881
− 5	− 101	1,535	87,631	− 51,419	− 5,755	229,733	1,698,095	− 98,945,651
− 3	− 103	931	93,771	− 31,809	− 6,693	146,349	2,255,127	− 65,594,781
− 1	− 104	312	96,876	− 10,764	− 7,176	50,232	2,549,274	− 22,943,466
41,650	433,160	770,715,400	372,255,538,2 (2)	372,255,538,2 (2)	2,045,360,100	4,344,753,924 (3)	259,407,366,7(5)	56 1,453,112,6(8)
1	6	2	1	9	924	132	99	11

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φ_1	φ_2	φ_3	φ_4	φ_5	φ_6	φ_7	φ_8	φ_9
− 25	1,225	− 4,900	46,060	− 75,670	378,350	− 378,350	16,269,050	− 22,776,670
− 24	1,078	− 3,724	27,636	− 30,268	60,536	45,402	− 7,158,382	18,221,336
− 23	937	− 2,668	12,596	2,162	− 127,558	235,658	− 13,851,934	22,404,806
− 22	802	− 1,727	611	23,782	− 218,362	273,493	− 11,481,301	11,248,886
− 21	673	− 896	− 8,634	36,547	− 239,131	221,007	− 5,268,403	− 2,681,179
− 20	550	− 170	− 15,440	42,214	− 212,440	124,295	1,459,205	− 13,018,336
− 19	433	456	− 20,094	42,351	− 156,657	16,131	6,801,009	− 17,484,617
− 18	322	987	− 22,869	38,346	− 86,394	− 81,621	9,893,913	− 16,290,722
− 17	217	1,428	− 24,024	31,416	− 12,936	− 155,856	10,592,322	− 10,959,662
− 16	118	1,784	− 23,804	22,616	55,352	− 200,324	9,210,326	− 3,498,672
− 15	25	2,060	− 22,440	12,848	112,640	− 213,960	6,318,770	4,147,998
− 14	− 62	2,261	− 20,149	2,870	155,270	− 199,435	2,590,385	10,424,850
− 13	− 143	2,392	− 17,134	− 6,695	181,415	− 161,915	− 1,313,455	14,321,385
− 12	− 218	2,458	− 13,584	− 15,350	190,760	− 108,015	− 4,820,395	− 15,403,680
− 11	− 287	2,464	− 9,674	− 22,715	184,205	− 44,935	− 7,494,835	13,759,515
− 10	− 350	2,415	− 5,565	− 28,518	163,590	20,235	− 9,061,005	9,889,082
− 9	− 407	2,316	− 1,404	− 32,586	131,442	81,054	− 9,406,254	4,567,562
− 8	− 458	2,172	2,676	− 34,836	90,744	132,126	− 8,569,038	− 1,299,448
− 7	− 503	1,988	6,556	− 35,266	44,726	169,366	− 6,715,702	− 6,811,538
− 6	− 542	1,769	10,131	− 33,946	− 3,322	190,149	− 4,109,761	− 11,188,418
− 5	− 575	1,520	13,310	− 31,009	− 50,215	193,355	− 1,076,995	− 13,856,459
− 4	− 602	1,246	16,016	− 26,642	− 93,016	179,323	2,030,717	− 14,502,656
− 3	− 623	952	18,186	− 21,077	− 129,157	149,727	4,870,289	− 13,094,921
− 2	− 638	643	19,771	− 14,582	− 156,538	107,387	7,138,901	− 9,870,266
− 1	− 647	324	20,736	− 7,452	− 173,604	56,028	8,600,298	− 5,294,646
0	− 650	0	21,060	− 0	− 179,400	0	9,104,550	0
11,050	17,218,110	221,375,700	17,803,525,74(1)	47,861,426,34 (1)	1,282,440,783 (3)	1,465,091,440 (3)	3,279,650,279 (6)	8,216,387,014 (6)
2	1	4	5	28	42	264	33	110

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φ_1	φ_2	φ_3	φ_4	φ_5	φ_6	φ_7	φ_8	φ_9
− 51	425	− 4,165	3,570	− 55,930	1,286,390	− 19,295,850	1,286,390	− 55,314,770
− 49	375	− 3,185	2,170	− 23,030	227,010	1,891,750	− 529,690	42,299,530
− 47	327	− 2,303	1,022	658	− 408,618	11,638,046	− 1,074,514	54,013,246
− 45	281	− 1,515	102	16,638	− 724,270	13,834,638	− 918,850	28,912,426
− 43	237	− 817	− 613	26,273	− 807,507	11,481,301	− 455,101	− 3,858,089
− 41	195	− 205	− 1,145	30,791	− 731,193	6,822,605	63,227	− 29,154,731
− 39	155	325	− 1,515	31,291	− 554,947	1,471,665	488,063	− 41,186,561
− 37	117	777	− 1,743	28,749	− 326,529	− 3,478,407	748,743	− 39,961,147
− 35	81	1,155	− 1,848	24,024	− 83,160	− 7,354,776	828,870	− 28,647,202
− 33	47	1,463	− 1,848	17,864	147,224	− 9,816,312	747,542	− 11,694,342
− 31	15	1,705	− 1,760	10,912	344,784	− 10,775,600	544,454	6,432,438
− 29	− 15	1,885	− 1,600	3,712	496,656	− 10,330,960	268,406	22,029,618
− 27	− 43	2,007	− 1,383	− 3,285	595,895	− 8,707,905	− 31,225	32,553,915
− 25	− 69	2,075	− 1,123	− 9,715	640,485	− 6,209,465	− 310,465	36,738,945
− 23	− 93	2,093	− 833	− 15,295	632,415	− 3,174,805	− 534,565	34,512,075
− 21	− 115	2,065	− 525	− 19,817	576,821	54,435	− 680,029	26,780,937
− 19	− 135	1,995	− 210	− 23,142	481,194	3,160,650	− 735,186	15,147,142
− 17	− 153	1,887	102	− 25,194	354,654	5,870,202	− 699,618	1,593,682
− 15	− 169	1,745	402	− 25,954	207,290	7,967,346	− 582,730	− 11,817,658
− 13	− 183	1,573	682	− 25,454	49,566	9,302,722	− 401,722	− 23,211,838
− 11	− 195	1,375	935	− 23,771	− 108,207	9,796,985	− 179,197	− 31,110,409
− 9	− 205	1,155	1,155	− 21,021	− 256,333	9,440,145	59,387	− 34,574,539
− 7	− 213	917	1,337	− 17,353	− 386,127	8,287,189	288,239	− 33,276,721
− 5	− 219	665	1,477	− 12,943	− 490,245	6,450,557	483,575	− 27,502,411
− 3	− 223	403	1,572	− 7,988	− 562,948	4,090,044	625,646	− 18,087,706
− 1	− 225	135	1,620	− 2,700	− 600,300	1,400,700	700,350	− 6,303,150
46,852	2,108,340	162,342,180	108,228,120	26,358,466,68 (1)	14,876,313,08 (4)	3,803,377,377 (6)	20,338,916,46 (4)	47,733,295,98 (7)
1	3	5	70	42	14	6	495	55

Appendix II.

Some Useful Relations.

$$x^{(1)} = x$$
$$x^{(2)} = x\,(x-1)$$
$$x^{(3)} = x\,(x-1)\,(x-2)$$
$$x^{(4)} = x\,(x-1)\,(x-2)\,(x-3)$$
$$x^{(5)} = x\,(x-1)\,(x-2)\,(x-3)\,(x-4)$$
$$x^{(6)} = x\,(x-1)\,(x-2)\,(x-3)\,(x-4)\,(x-5)$$

$$x^{[1]} = x$$
$$x^{[2]} = (x^2 - \tfrac{1}{4})$$
$$x^{[3]} = x\,(x^2 - 1)$$
$$x^{[4]} = (x^2 - \tfrac{1}{4})\,(x^2 - {}^9/_4)$$
$$x^{[5]} = x\,(x^2 - 1)\,(x^2 - 4)$$
$$x^{[6]} = (x^2 - \tfrac{1}{4})\,(x^2 - {}^9/_4)\,(x^2 - {}^{25}/_4)$$

$$\mu x^{[1]} = x$$
$$\mu x^{[2]} = x^2$$
$$\mu x^{[3]} = x\,(x^2 - \tfrac{1}{4})$$
$$\mu x^{[4]} = x^2\,(x^2 - 1)$$
$$\mu x^{[5]} = x\,(x^2 - \tfrac{1}{4})\,(x^2 - {}^9/_4)$$
$$\mu x^{[6]} = x^2\,(x^2 - 1)\,(x^2 - 4)$$

$$x = \mu x^{[1]} = x^{[1]}$$
$$x^2 = \mu x^{[2]}$$
$$x^3 = x^{[3]} + x^{[1]}$$
$$x^4 = \mu x^{[4]} + \mu x^{[2]}$$
$$x^5 = x^{[5]} + 5x^{[3]} + x^{[1]}$$
$$x^6 = \mu x^{[6]} + 5\mu x^{[4]} + \mu x^{[2]}$$
$$x^7 = x^{[7]} + 14x^{[5]} + 21x^{[3]} + x^{[1]}$$
$$x^8 = \mu x^{[8]} + 14\mu x^{[6]} + 21\mu x^{[4]} + \mu x^{[2]}$$

$$x^{(1)} = \mu x^{[1]} = x^{[1]}$$
$$x^{(2)} = \mu x^{[2]} - x^{[1]}$$
$$x^{(3)} = x^{[3]} - 3\mu x^{[2]} + 3x^{[1]}$$
$$x^{(4)} = \mu x^{[4]} - 6x^{[3]} + 12\mu x^{[2]} - 12x^{[1]}$$
$$x^{(5)} = x^{[5]} - 10\mu x^{[4]} + 40x^{[3]} - 60\mu x^{[2]} + 60x^{[1]}$$
$$x^{(6)} = \mu x^{[6]} - 15x^{[5]} + 90\mu x^{[4]} - 300x^{[3]} + 360\mu x^{[2]} - 360x^{[1]}$$

APPENDIX III.

Binomial Coefficients.

n	$\binom{n}{2}$	$\binom{n}{3}$	$\binom{n}{4}$	$\binom{n}{5}$	$\binom{n}{6}$	$\binom{n}{7}$	$\binom{n}{8}$	$\binom{n}{9}$	$\binom{n}{10}$
2	1								
3	3	1							
4	6	4	1						
5	10	10	5	1					
6	15	20	15	6	1				
7	21	35	35	21	7	1			
8	28	56	70	56	28	8	1		
9	36	84	126	126	84	36	9	1	
10	45	120	210	252	210	120	45	10	1
11	55	165	330	462	462	330	165	55	11
12	66	220	495	792	924	792	495	220	66
13	78	286	715	1287	1716	1716	1287	715	286
14	91	364	1001	2002	3003	3432	3003	2002	1001
15	105	455	1365	3003	5005	6435	6435	5005	3003
16	120	560	1820	4368	8008	11440	12870	11440	8008
17	136	680	2380	6188	12376	19448	24310	24310	19448
18	153	816	3060	8568	18564	31824	43758	48620	43758
19	171	969	3876	11628	27132	50388	75582	92378	92378
20	190	1140	4845	15504	38760	77520	1 25970	1 67960	1 84756
21	210	1330	5985	20349	54264	1 16280	2 03490	2 93930	3 52716
22	231	1540	7315	26334	74613	1 70544	3 19770	4 97420	6 46646
23	253	1771	8855	33649	1 00947	2 45157	4 90314	8 17190	11 44066
24	276	2024	10626	42504	1 34596	3 46104	7 35471	13 07504	19 61256

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